Clean Water Act §319(h) Nonpoint Source Grant Program

Bacterial Source Tracking to Support Adaptive Management of the Arroyo Colorado Watershed Protection Plan

TSSWCB Project # 12-10
Revision 0

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

prepared by

Texas A&M AgriLife Research
Texas Water Resources Institute

and the

University of Texas at Brownsville, Department of Chemistry and Environmental Sciences
and the
University of Texas Health Science Center at Houston School of Public Health, El Paso

Effective Period: Upon EPA Approval through October 31, 2014

(with annual updates required)

Questions concerning this quality assurance project plan should be directed to:

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Section A1 Approval Sheet

Quality Assurance Project Plan (QAPP) for the *Bacterial Source Tracking to Support Adaptive Management of the Arroyo Colorado Watershed Protection Plan.*

United States Environmental Protection Agency (USEPA), Region VI

Name: Curry Jones
Title: USEPA Chief State/Tribal Programs Section

Signature: _____ Date:_____

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Name: Ashley Wendt
Title: TSSWCB Project Manager (PM)

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Name: Mitch Conine
Title: TSSWCB Quality Assurance Officer (QAO)

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Texas A&M AgriLife Research – Texas Water Resources Institute (TWRI)

Name: Kevin Wagner
Title: Associate Director/Project Lead

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Name: Lucas Gregory
Title: TWRI QAO

Signature: _____ Date:_____

Name: Ashley Gregory
Title: TWRI PM

Signature: _____ Date:_____

University of Texas at Brownsville Chemistry and Environmental Sciences (UTB)

Name: Jude Benavides
Title: Associate Professor/Project Co-Lead

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Brownsville Public Utilities Board – Analytical Laboratory (B-PUB)

Name: Lee Roy Atkinson
Title: Laboratory Manager

Signature: _____ Date:_____

Name: Michael McCall
Title: Quality Assurance Specialist

Signature: _____ Date:_____

University of Texas Health Science Center at Houston, School of Public Health, El Paso Regional Campus (UTSPH-EP)

Name: George D. Di Giovanni
Title: Professor/Project Co-Lead

Signature: _____ Date:_____

Section A2: Table of Contents

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List of Acronyms and Abbreviations

ACWP	Arroyo Colorado Watershed Partnership
ACWPP	Arroyo Colorado Watershed Protection Plan
ANRA	Angelina-Neches River Authority
AWRL	Ambient Water Reporting Limits
BMP	best management practice
BSLC	Bacteria Source Load Calculator
BST	bacterial source tracking
CFU	colony forming unit
CAR	corrective action report
COC	chain of custody form
CMS	Coordinated Monitoring Schedule
CRP	Clean Rivers Program
CWA	Clean Water Act
DMRG	TCEQ Data Management Reference Guide
DQO	data quality objectives
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reaction
ERIC-RP	ERIC-PCR and RiboPrinting composite method
LCS	Laboratory Control Sample
LCSD	Laboratory control sample duplicate
LOQ	limit of quantification
LM	Laboratory Manager
mTEC	membrane thermotolerant *E. coli*
MS	matrix spike
MS4	municipal separate storm sewer systems
NIST	National Institute of Standards and Technology
OSSFs	On-site Sewage Facilities
PM	Project Manager
QA	Quality assessment
QAPP	quality assurance project plan
QAO	Quality Assurance Officer
QC	quality control
QM	quality manual
QPR	quarterly progress report
RPD	relative percent difference
SLOC	station location
SOP	Standard Operating Procedure
SWAT	Soil and Water Assessment Tool
SWCD	Soil and Water Conservation District
SWQMIS	Surface Water Quality Management Information System
TCEQ	Texas Commission on Environmental Quality
TMDL	total maximum daily load
TPDES	Texas Pollutant Discharge Elimination System

TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas A&M AgriLife Research-Texas Water Resources Institute
USDA-ARS	United States Department of Agriculture-Agricultural Research Service
USDA–APHIS	United States Department of Agriculture – Animal Plant Health Inspection Service
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UTB	University of Texas at Brownsville
B-PUB	Brownsville – Public Utilities Board
UTSPH-EP	University of Texas School of Public Health, El Paso Regional Campus
WPP	watershed protection plan

Section A3: Distribution List

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

United States Environmental Protection Agency, Region VI

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Dallas, TX 75202-2733

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University of Texas Health Science Center at Houston, School of Public Health, El Paso Regional Campus

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El Paso, TX 79902

Name: George D. Di Giovanni
Title: Professor; Project Co-Lead

Section A4: Project/Task Organization

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

USEPA – United States Environmental Protection Agency, Dallas, Texas. Provides project oversight and funding at the federal level.

Henry Brewer, USEPA Texas Nonpoint Source Project Officer

Responsible for overall performance and direction of the project at the federal level. Ensures that the project assists in achieving the goals of the clean water act (CWA). Reviews and approves the QAPP, project progress, and deliverables.

TSSWCB – Texas State Soil and Water Conservation Board, Temple, Texas. Provides project overview at the State level.

Ashley Wendt, TSSWCB PM

Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified. Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants.

Mitch Conine, TSSWCB QAO

Reviews and approves QAPP and any amendments or revisions. Responsible for verifying that the QAPP is followed by project participants. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures. Determines that the project meets the requirements for planning, quality assessment (QA), quality control (QC), and reporting under the TSSWCB CWA §319(h) Nonpoint Source Grant program.

TWRI – Texas A&M AgriLife Research - Texas Water Resources Institute, College Station, Texas. Responsible for general project oversight, coordination administration, reporting and development of data quality objectives (DQOs) and a QAPP.

Kevin Wagner, TWRI Associate Director; Project Lead

Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract and in the project QAPP; assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB PM.

Lucas Gregory, TWRI QAO

Responsible for determining that the QAPP meets the requirements for planning, QA and QC. Conducts audits of field and laboratory systems and procedures. Responsible for maintaining the official, approved QAPP, as well as conducting quality assurance

audits in conjunction with TSSWCB personnel. Responsible for coordinating with the TSSWCB QAO to resolve QA-related issues. Notifies the TSSWCB PM of particular circumstances which may adversely affect the quality of data. Responsible for validation and verification of all data collected according with procedures listed in this document and acquired data procedures after each task is performed.

Ashley Gregory, TWRI PM

Responsible for supporting the development and ensuring the timely delivery of project deliverables, ensuring cooperation between project partners, providing fiscal oversight and completing project reporting.

UTB – University of Texas at Brownsville, Department of Chemistry and Environmental Sciences, Brownsville, Texas. Responsible for conducting water quality analysis, maintaining a water quality database and transmitting project data for inclusion in the Surface Water Quality Monitoring Information System (SWQMIS) database.

Jude Benavides, UTB Associate Professor; Project Co-Lead

Responsible for overseeing and carrying out scheduled routine monitoring, sample collection, and coordinating delivery of collected samples with B-PUB. This involves ensuring that field personnel involved in the collection have adequate training and thorough knowledge of the QAPP and all SOPs specific to the task performed. Responsible for all field operations ensuring that all QA/QC requirements are met, documentation related to the data collection are complete and adequately maintained and that results are reported accurately. Responsible for ensuring that corrective action are implemented, documented, reported and verified.

B-PUB – Brownsville Public Utilities Board - Analytical Laboratory, Brownsville, Texas. Responsible for enumeration of *E. coli* and enterococci bacteria in water samples and shipment of Method 1603 *E. coli* plates to UTSPH-EP for BST analysis.

Lee Roy Atkinson, B-PUB Laboratory Manager

Monitors all sample analysis within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems. Ensures that adequate training and thorough knowledge of the QAPP and all SOPs specific to the analysis performed are fully understood. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the data collection and analysis are complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Ensures that proper shipping procedures are utilized in sending prepared samples to UTSPH-EP.

Michael McCall, B-PUB Quality Assurance Specialist (QAS)

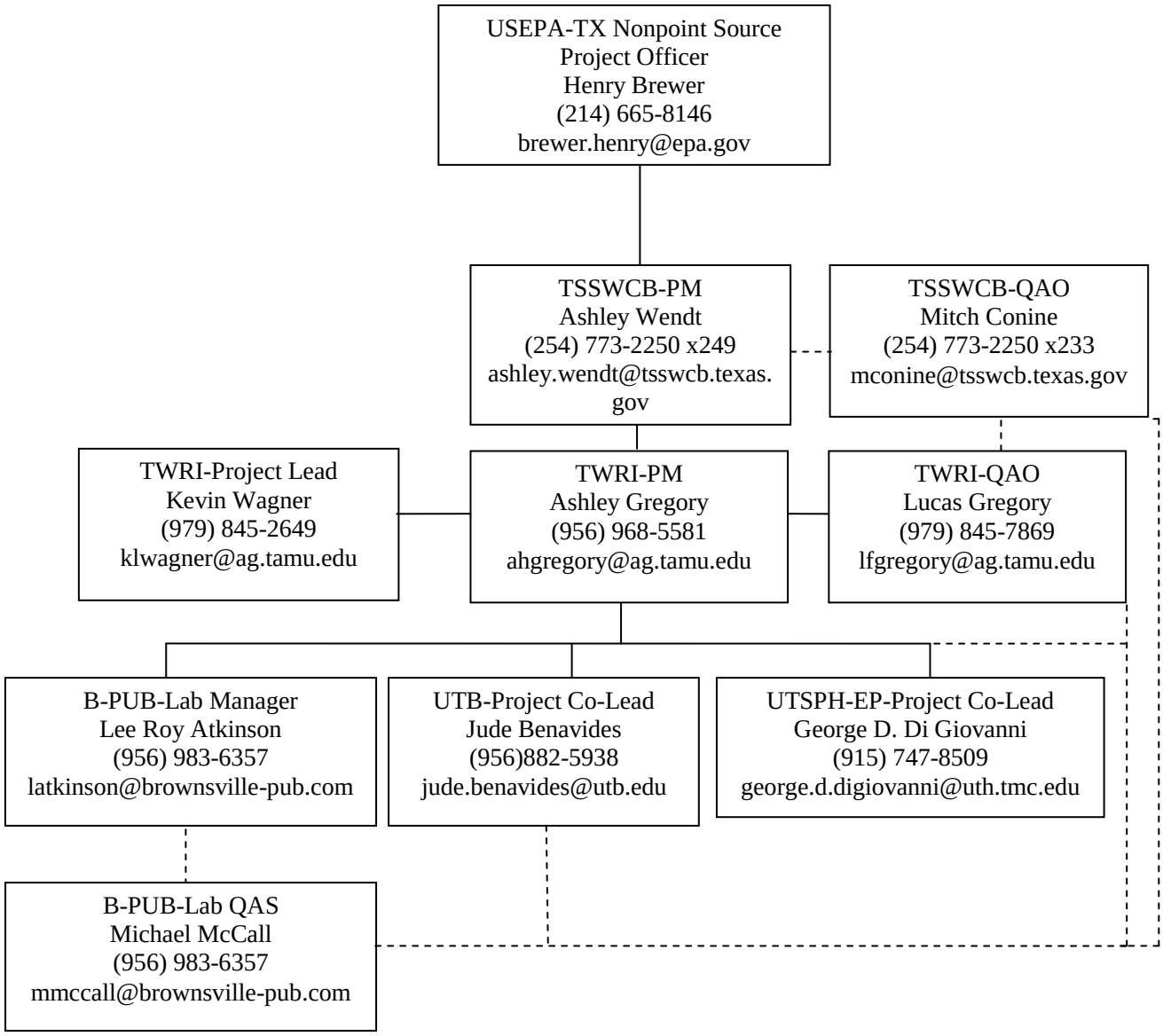
Responsible for processing bacteria samples in preparation of BST analysis and related activities, as well as enumeration of bacteria. This includes ensuring the laboratory personnel involved in processing samples have adequate training and knowledge of the QAPP, and all standard operating procedures (SOPs) specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

UTSPH-EP – University of Texas Health Science Center at Houston, School of Public Health, El Paso Regional Campus, El Paso, Texas. Responsible for bacterial source tracking.

George D. Di Giovanni, UTSPH-EP Professor; Project Co-Lead

Responsible for performing BST analysis and related activities. This includes ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and all standard operating procedures (SOPs) specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

Figure A4-1. Project Organization Chart



Section A5: Problem Definition/Background

The Arroyo Colorado Watershed is located in the Lower Rio Grande Valley of South Texas and flows through the middle of Hidalgo and Cameron counties. The lower 16 miles of the Arroyo Colorado is the boundary between Cameron and Willacy counties. The Arroyo Colorado drainage area is a subwatershed of the Nueces-Rio Grande Coastal Basin, also known as the Lower Laguna Madre Watershed. The streams of the Nueces-Rio Grande Coastal Basin, including the Arroyo Colorado, drain to the Laguna Madre, which is considered to be one of the most productive hypersaline lagoon systems in the world. The Lower Rio Grande Valley comprises the northern part of the Rio Grande Delta, a broad fluviodeltaic plain laid down over tens of thousands of years by the ancestral Rio Grande. Just as the Rio Grande is the major source of freshwater for the Lower Rio Grande Valley, the Arroyo Colorado serves as the main drainage stream for this area of Texas.

The Arroyo Colorado currently has low dissolved oxygen levels within the tidal segment, not meeting the aquatic life use designated by the State of Texas and described in the Water Quality Standards. This has been the case for every 303(d) list prepared by the state since 1996. In addition, bacteria has always been a parameter of concern and as of 2006, the Arroyo became impaired due to high bacteria levels. There are many challenges associated with restoring water quality in the Arroyo Colorado Watershed. The watershed is one of the most productive agricultural areas in the state; however, it also has one of the fastest growing populations of any region in the state as well, which increases the threat for bacterial impairments.

Surface water irrigation on consumptive crops is widely used. The bacteria impairment not only poses a human health threat through contact recreation, but also potentially through consuming food that is grown with this water.

Previous work conducted in this area has laid the ground work and produced outcomes that will be incorporated into this effort. Specifically, the TSSWCB-funded project (06-10) entitled *Arroyo Colorado Agricultural Nonpoint Source Assessment* utilized the Soil and Water Assessment Tool (SWAT) model to simulate flow and nutrient loadings to the Arroyo Colorado. These data provide critical flow and nutrient information that will aid in the development of best management practices (BMPs) to address bacteria and nutrient loadings and develop estimated load reductions that will be incorporated into the revision of the Arroyo Colorado Watershed Protection Plan (WPP). Additionally, other efforts are ongoing with a range of foci from sustaining the partnership to educating landowners on available financial incentive programs to implement agricultural BMPs.

TCEQ is funding a project to revise the Arroyo Colorado WPP. Initially written with implementation scheduled through 2015, the Arroyo Colorado WPP focused primarily on nutrients, but through adaptive management, the bacteria impairment has become an issue stakeholders are prepared to address in the next phase of the plan.

Although data collected in the watershed tend to justify the currently listed impairment, this data remains limited and additional data is needed to accurately calculate bacteria loading rates and the most likely sources of bacterial contamination. The needs for a bolstered data set and comprehensive data analysis arise as management options are considered. Without adequate data, uncertainty increases in properly identifying the sources of contamination in the watershed, while comprehensive data analysis is needed to hone in on potential sources of water pollutants. Collecting 12 months of additional water quality and stream flow data along with input from local stakeholders will provide much needed information that will enable more accurate watershed pollutant source assessments and the revision of a focused and effective Arroyo Colorado WPP.

Section A6: Project Goals and Task Description

Through this project, a water quality monitoring regime will be employed that will help decision makers make appropriate recommendations for addressing the bacteria impairment in the revision of the Arroyo Colorado WPP.

UTB will conduct routine ambient monitoring at 10 locations (shown in Table A6.1 and Figure A6.1) as identified in the ACWPP monthly, collecting field and bacteria parameter groups. The sampling period extends over 12 months. Total number of samples scheduled for collection through this subtask is 120 *E. coli* and 120 *Enterococcus* samples. UTB will deliver water samples to the NELAP–accredited B-PUB for *E. coli* and *Enterococcus* enumeration. *E. coli* will be enumerated using EPA Method 1603 with the plates saved for BST analysis. *Enterococcus* will be enumerated only (no BST analysis) using the SM-9223 B Chromogenic Substrate Test IDEXX Enterolert method.

Field parameters are pH, temperature, conductivity, and dissolved oxygen. These parameters will be measured with a hand-held YSI 556 multiparameter meter or a YSI EXO1 multiparameter sonde. Flow parameters are flow collected by gage, electrical, mechanical or Doppler, including severity. An instantaneous water velocity measurement will be made at all non-tidal stations using a Marsh-McBirney Flo-Mate Portable Velocity Flowmeter. Velocity measurements will follow protocols outlined in the TCEQ SWQM Procedures Manual, Volume 1 (August, 2012) and provided relevant updates located on the TCEQ website. Parameter code 00061 – Flow (Stream Discharge) will be utilized unless field conditions are considered unsafe for personnel (based on either excessive stream velocity, overall depth, floating debris or trash, or other unsafe conditions as determined by senior field personnel present). In this event, parameter code 74069 Flow Estimate will be utilized. Multiple site visits to all stations over the summer of 2013 and early fall 2013 suggest that parameter code 00061 will be the likely method followed for the three most upstream stations. Site visits to the mid-stream stations (Station ID 13081, 13080, and 16445) suggest that the common presence of snags, debris, and other hazards may necessitate the use of a flow estimate.

B-PUB will store Method 1603 modified mTEC plates for *E. coli* at 4ºC for shipment to UTSPH-EP. B-PUB will coordinate the shipment of these samples with UTSPH-EP such that they are received in El Paso within 3 days following enumeration.

TWRI will collaborate with UTSPH-EP, local stakeholders and technical experts to design a source survey that characterizes possible sources of bacteria loadings in the study area. The source survey will represent warm and cool seasons and low and high flow conditions. Results from the source survey will be used by UTSPH-EP to assess the adequacy of the Texas *E. coli* BST Library and to guide the collection of known source fecal samples.

TWRI will work with others to collect 200 known source fecal samples from the study area to supplement the Texas *E. coli* BST Library. Fecal samples will be stored at 4°C and shipped to UTSPH-EP for *E. coli* isolation and analysis. TWRI will coordinate the shipment of these samples with UTSPH-EP such that they are received in El Paso within 3 days of collection.

To assess and identify different sources contributing to bacterial loadings, UTSPH-EP will conduct library-dependent BST and analyze *E. coli* isolates using the Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR) and RiboPrinting composite method. Isolates will be screened using ERIC-PCR and the non-clonal isolates will be further analyzed using RiboPrinting. An estimated 1,000 fecal *E. coli* isolates (up to 5 per fecal sample) will be archived. An estimated 600 isolates (3 per fecal sample) will be screened using ERIC-PCR, and an estimated 300 non-clonal fecal isolates will be analyzed using RiboPrinting.

Using the Method 1603 plates prepared and enumerated by B-PUB, UTSPH-EP will isolate and confirm up to 960 *E. coli* isolates (8 isolates per water sample) for archival. UTSPH-EP will conduct library-dependent BST to analyze 600 of the *E. coli* isolates from the 120 water samples (5 isolates per water sample) using ERIC-RP.

UTSPH-EP will analyze data to assess different sources contributing to bacterial loading in the Arroyo Colorado watershed.

UTB will maintain a database for housing all environmental water quality data collected through the project. UTB will maintain a database of field parameters data collected under the project and transmit this data to TWRI for inclusion into the master database. Data from the routine monitoring will be transferred for inclusion in the TCEQ SWQMIS at least quarterly.

Table A6.1. Water Quality Monitoring Stations

Station ID	Description
13086	Arroyo Colorado at FM 336 South of McAllen
13084	Arroyo Colorado at US 281 South of Pharr
13082	Arroyo Colorado at FM 493 South of Donna
13080	Arroyo Colorado at FM 506 South of La Feria
13079	Arroyo Colorado at U.S. 77 in Southwest Harlingen
13074	Arroyo Colorado at Low Water Bridge at Port Harlingen
13072	Arroyo Colorado Tidal FM 106 Bridge at Rio Hondo
13073	Arroyo Colorado Tidal at Camp Perry North of Rio Hondo
13559	Arroyo Colorado Tidal at Marker 27 (Mile 15) 0.5 Mile North of the Point Where Channel Becomes Boundary Between Willacy and Cameron Counties
13782	Arroyo Colorado Tidal Near CM 16 at Arroyo City, KM 10.9

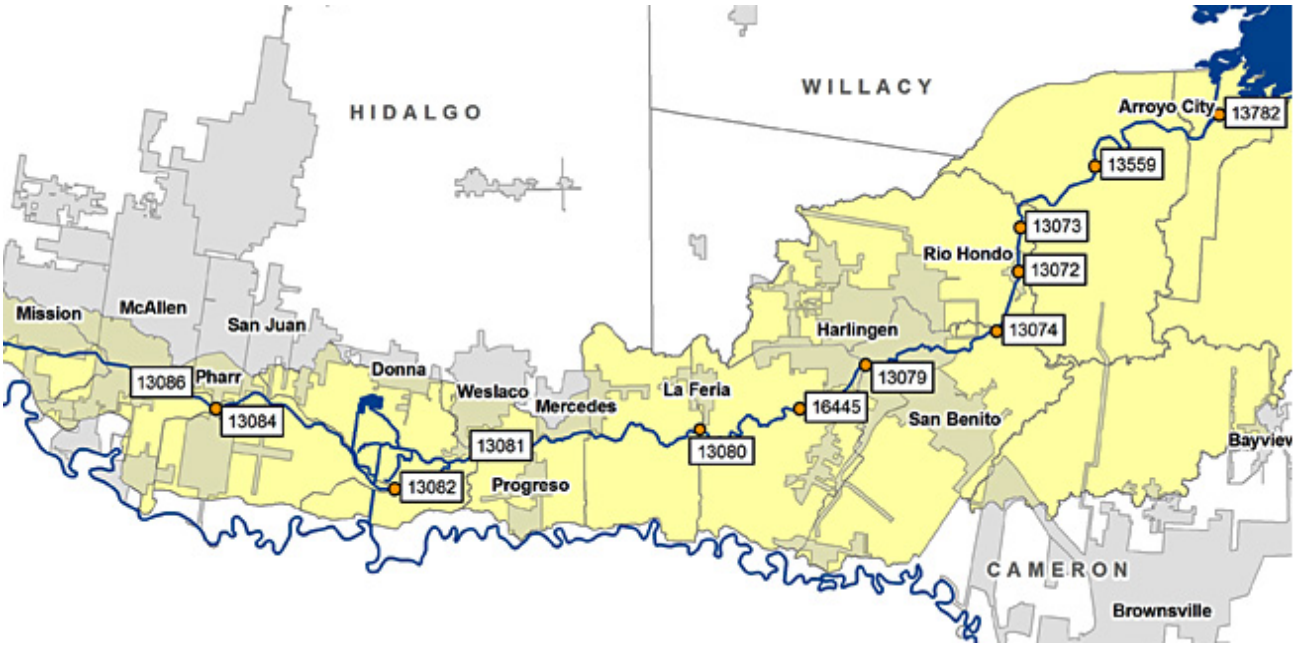


Figure A6.1. Arroyo Colorado Watershed Map and Monitoring Stations (Stations 13081 and 16445 shown will not be monitored through this project)

The purpose of this QAPP is to clearly delineate the QA policy, management structure, and procedures, which will be used to implement the QA requirements necessary to conduct a watershed source survey under Task 4; collect and monitor water quality throughout the Arroyo Colorado watershed under Task 3, and to analyze water and fecal samples collected throughout the watershed utilizing BST under Task 5.

Table A6.2. Project Plan Milestones

Task	Project Milestones	Agency	Start	End
2.1	Develop QAPP for tasks 3 - 5	TWRI UTB, UTSPH-EP, B-PUB	Month 1	Month 19
2.2	Submit revisions and amendments as necessary	TWRI UTB, UTSPH-EP, B-PUB	Month 20	Month 36
3.1	Conduct routine ambient monitoring at 10 locations identified in ACWPP	UTB	Month 20	Month 32
3.1	Enumerate and prepare samples for BST analysis	B-PUB	Month 20	Month 24
3.2	Store Method 1603 plates and ship samples to UTSPH-EP	B-PUB	Month 20	Month 27
3.3	Maintain a database for all data collected and acquired from Brownsville PUB and transmit to TWRI to upload into SWQMIS	UTB	Month 20	Month 33
4.1	Host meeting of local experts to identify targeted sources of bacteria	TWRI	Month 20	Month 27
4.1	Conduct Source Survey as designed	TWRI	Month 20	Month 27
4.2	Collect known source fecal samples from the area and ship samples to UTSPH-EP	TWRI	Month 20	Month 27
5.1	Supplement the Texas *E. coli* BST Library with known fecal samples	UTSPH-EP	Month 20	Month 31
5.2	Conduct library-dependent BST	UTSPH-EP	Month 20	Month 31
5.3	Analyze data to assess different sources contributing to bacteria loadings	UTSPH-EP	Month 32	Month 36

Surface Water Quality Monitoring

UTB will be responsible for the collection and transport of all water quality data and samples to the respective lab (B-PUB) within appropriate sample holding times and in accordance with this QAPP. Sampling will be conducted routinely at the sampling sites designated in Table

A6.1.

For BST analysis purposes, the B-PUB will receive and process water samples using standard microbiological methods as previously used in TSSWCB and TCEQ BST projects. *E. coli* will be isolated from water samples using USEPA Method 1603 and modified membrane thermotolerant *E. coli* (mTEC) medium. After enumeration, B-PUB will periodically ship or arrange to deliver the Method 1603 plates to UTSPH-EP for BST analyses. Samples will be shipped in cooler boxes; *E. coli* samples will be packaged with blue ice. All sample shipments will be accompanied by proper notification to UTSPH-EP personnel.

Fecal specimens or domestic sewage samples collected by TWRI or project partners and sent to UTSPH-EP will be streaked (resuspended in buffer if necessary) onto modified mTEC medium. The use of modified mTEC medium for isolation of *E. coli* from both water and source samples will help avoid selection of different types of *E. coli* due to different media. Inoculated plates will be incubated at 35±0.5^{o}C for 2 hours to resuscitate stressed bacteria, then incubated at 44.5±0.2^{o}C for approximately 20 to 24 hours. The modified mTEC method is a single-step method that uses one medium and does not require testing using any other substrate. The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with 4-methylumbelliferyl-β-D-glucuronide (MUG) observed by ultraviolet light (UV) fluorescence. *E. coli* colonies from the modified mTEC medium will be picked and streaked for purity on nutrient agar with MUG (NA-MUG) to confirm glucuronidase activity and culture purity for both water and source sample isolates.

BST Analysis

Identification of Sources

New data, of known and specified quality, will be collected and analyzed to differentiate and quantify the relative contributions of livestock, wildlife, and human *E. coli* sources to the Arroyo Colorado. This assessment and differentiation between bacteria sources will utilize the Texas *E. coli* BST Library coordinated by UTSPH-EP.

600 *E. coli* isolates from 120 different water samples (5 isolates per water sample) collected from across the study area will be analyzed by UTSPH-EP using the ERIC-PCR and RiboPrinting BST methods described below and compared with isolates from the previously developed Texas *E. coli* BST Library. An experimental approach flow diagram is presented in Figure A6.2.

Limited Library Dependent BST

Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR) has a moderately high ability to resolve different closely related bacterial strains (Versalovic, Schneider et al. 1994). Consumable costs for ERIC-PCR are inexpensive and labor costs for sample processing and data analyses are moderate. ERIC-PCR is a genetic

fingerprinting method used in previous BST studies as well as many microbial ecology and epidemiological studies. ERIC elements are repeat DNA sequences found in varying numbers and locations in the genomes of different bacteria such as *E. coli*. The PCR is used to amplify the DNA regions between adjacent ERIC elements. This generates a DNA banding pattern or fingerprint which looks similar to a barcode pattern. Different strains of *E. coli* bacteria have different numbers and locations of ERIC elements in their bacterial genomes, and therefore, have different ERIC-PCR fingerprints. ERIC-PCR is useful as a screening technique for library development because of its moderate cost and moderately high ability to resolve different strains of the same species of bacteria. Though ERIC-PCR banding patterns for isolates tend to be generally stable, differences in fingerprint image processing and PCR protocols between laboratories may result in reduced between-laboratory reproducibility and pose a challenge to generating a composite library in multiple laboratories. Rigorous QA/QC, standardized protocols for PCR and image processing, and adequate training of personnel is crucial for generation of comparable data (Bacteria TMDL Task Force Final Report; TWRI TR-341).

Ribotyping is a genetic fingerprinting method used in previous BST studies and many microbial ecology and epidemiological studies. In general, an endonuclease enzyme (*Hind* III) selectively cuts *E. coli* DNA wherever it recognizes a specific DNA sequence. The resulting DNA fragments are separated by size and probed for fragments containing particular conserved ribosomal RNA gene sequences, which results in DNA banding patterns or fingerprints that look similar to barcodes. Different strains of *E. coli* bacteria have differences in their DNA sequences and different numbers and locations of enzyme cutting sites, and therefore have different ribotyping fingerprints. The DuPont Qualicon RiboPrinter Microbial Characterization System allows automation of the ribotyping ('RiboPrinting'). DNA patterns of those isolates will be compared to the Texas *E. coli* BST Library of *E. coli* isolates from known animal and human sources collected throughout Texas. Water isolates will be identified to cattle, other livestock, avian wildlife, non-avian wildlife, domestic sewage, and pet sources (six-way split), as well as a more general three-way split between livestock, domestic sewage and wildlife.

Section A7: Quality Objectives and Criteria for Data Quality

The goal of this project is to support the update of a WPP for the Arroyo Colorado watershed by conducting a series of water quality evaluations including, but not limited to routine ambient monitoring and stream flow monitoring. Data gathered under these efforts will be used to support characterization in the WPP, and inform the stakeholder process.

The purpose of collecting routine ambient sampling under this project is to support the stakeholder decision-making as part of the WPP process. UTB and TWRI will provide TSSWCB with ambient water quality data on a quarterly basis for inclusion in TCEQ's SWQMIS. Routine water quality monitoring is needed for conducting water quality assessments in accordance with TCEQ's *Guidance for Assessing and Reporting Surface Water Quality in Texas*. The measurement performance specifications to support the project objectives are specified in Table A7.1.

The purpose of conducting BST analysis is to better characterize bacteria so that stakeholders can make informed decisions on managing causes and sources.

The objective of this section is to ensure that data collected meets the data quality objectives (DQOs) of the project. One objective is to identify specific sources of bacteria entering the Arroyo Colorado. Achievement of this objective will support decisions for implementation of appropriate best management practices (BMPs) in order to reduce fecal bacteria levels in the Arroyo Colorado watershed to comply with existing water quality standards. Ultimately, the results of this project will help watershed stakeholders make appropriate recommendations for addressing the bacteria impairment in the revision of the Arroyo Colorado WPP.

The following are actions that will be undertaken by this project to assess bacterial pollution within the Arroyo Colorado Watershed:

- Monitor water quality as related to bacterial pollution in Arroyo Colorado
- Determine the source(s) of the bacterial impairment using BST
- Contribute to the Texas *E. coli* BST Library

The measurement performance criteria to support the project objectives are specified in Table A7.1.

Surface Water Quality Monitoring

When sufficient flow (above 7Q2 or 0.1 cfs) is present, routine grab samples will be collected on a monthly basis. During routine sampling, measurements of dissolved oxygen (DO), conductivity, pH, salinity, stream flow, and water temperature will be obtained *in situ*. These parameters will be measured with a hand-held YSI 556 multiparameter meter or a YSI EXO1 multiparameter sonde. An instantaneous water velocity measurement will be made at all non-tidal stations using a Marsh-McBirney Flo-Mate Portable Velocity Flowmeter. These data will be logged on field data sheets (Appendix B), incorporated into a computer based database

maintained by UTB and transmitted to TWRI for inclusion in the master database that will be maintained.

Water samples collected will be transported to the B-PUB for bacteria enumeration and data logging. UTB will deliver water samples to B-PUB within designated holding times for respective analysis; Brownsville PUB will use designated methods outlined in Tables A7.1 and B2.1. Appropriate DQOs and QA/QC requirements for this analysis are also reported in Tables A7.1 and B2.1. Water samples will be processed for enumeration and later BST processing.

BST Analysis

The objective of this portion of the project is to assess potential bacteria sources and support adaptive management for updating the Arroyo Colorado Watershed Protection Plan by conducting BST. The measurement performance specifications to support the project objective are specified in Table A7.2. Laboratory measurement QC requirements and acceptability criteria are provided in Section B5.

Ambient Water Reporting Limits (AWRLs)
The AWRL establishes the reporting specification at or below which data for a parameter must be reported to be compared with freshwater screening criteria. The AWRLs specified in Table A7.1 are the program-defined reporting specifications for each analyte and yield data acceptable for the TCEQ's water quality assessment. A full listing of AWRLs can be found at http://www.tceq.texas.gov/waterquality/clean-rivers/qa/index.html. The limit of quantitation is the minimum level, concentration, or quantity of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence. The following requirements must be met in order to report results to the TSSWCB:

- The laboratory's LOQ for each analyte must be at or below the AWRL as a matter of routine practice
- The laboratory must demonstrate its ability to quantitate at its LOQ for each analyte by running an LOQ check standard for each analytical batch of samples analyzed.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria are provided in Section B5.

Precision
Precision is the degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. It is a measure of agreement among replicate measurements of the same property, under prescribed similar conditions, and is an indication of random error.

Field splits are used to assess the variability of sample handling, preservation, and storage, as well as the analytical process, and are prepared by splitting samples in the field. Control limits for field splits are defined in Section B5.

Laboratory precision is assessed by comparing replicate analyses of laboratory control samples in the sample matrix (e.g. deionized water, sand, commercially available tissue) or sample/duplicate pairs in the case of bacterial analysis. Precision results are compared against measurement performance specifications and used during evaluation of analytical performance. The ERIC-PCR and RiboPrinting BST techniques are qualitative assays, generating two different types of DNA fingerprints. Precision for ERIC-PCR and RiboPrinting will be determined using a control strain of *E. coli* (QC101). For ERIC-PCR and RiboPrinting, the data quality objective is 90% precision. Program-defined measurement performance specifications for precision are defined in Tables A7.1 and A7.2.

Table A7.1. Measurement Performance Specifications

Parameter	Units	Matrix	Method	Parameter Code	AWRL	Limit of Quantitation (LOQ)	Precision (RPD of LCS/LCSD)	Bias % Rec. of LCS	LOQ Check Standard % Rec	Lab
Field Parameters										
pH	pH/units	Water	EPA 150.1 & TCEQ SOP, V1	00400	NA	NA	NA	NA	NA	Field
DO	Mg/L	Water	SM 4500-O G & TCEQ SOP, V1	00300	NA	NA	NA	NA	NA	Field
Specific Conductance	µS/cm	Water	EPA 120.1 & TCEQ SOP, V1	00094	NA	NA	NA	NA	NA	Field
Temperature	Celsius	Water	SM 2250 B & TCEQ SOP, V1	00010	NA	NA	NA	NA	NA	Field
Days since last significant rainfall	Days	NA	TCEQ SOP, V1	72053	NA	NA	NA	NA	NA	Field
Total water depth	Meters	Water	TCEQ SOP, V2	82903	NA	NA	NA	NA	NA	Field
Flow measurement method	1-gage 2-electric 3-mechanical 4-weir/flume 5-doppler	Water	TCEQ SOP, V1	89835	NA	NA	NA	NA	NA	Field
Flow severity	1-no flow 2-low 3-normal 4-flood 5-high 6-dry	Water	TCEQ SOP, V1	01351	NA	NA	NA	NA	NA	Field
Present weather	1-clear 2-partly cloudy 3-cloudy 4-rain 5-other	NA	NA	89966	NA	NA	NA	NA	NA	Field
Bacteriological Parameters										
E. coli, modified mTEC**	CFU/100 mL	Water	EPA 1603	31648	1	1	3.27*∑Rlog/n	NA	NA	B-PUB
*Enterococcus***	MPN	Water	IDEXX	31701	1	1	NA	NA	NA	B-PUB

** Based on a range statistic as described in Standard Methods, 20th Edition, Section 9020-B, AQuality Assurance/Quality Control – Intralaboratory Quality Control Guidelines. This criterion applies to bacteriological duplicates with concentrations >10 MPN/ml100 ml or >10 organisms/ml100 ml.

Table A7.2. Measurement Performance Specifications for BST Analysis

Parameter	Method Type	Method	Method Description	Precision of Laboratory Duplicates	Bias	Percent Complete	Lab
E. coli ERIC-PCR	DNA/image matching	UTSPH-EP SOP	ERIC-PCR	90% identical*	90% correct*	90	UTSPH-EP
E. coli RiboPrinting	DNA/image matching	UTSPH-EP SOP	RiboPrinting	90% identical*	90% correct*	90	UTSPH-EP

*Accuracy and laboratory method precision for BST will be determined using an *E. coli* QC isolate

Bias

Bias is a statistical measurement of correctness and includes multiple components of systematic error. A measurement is considered unbiased when the value reported does not differ from the true value. Bias is determined through the analysis of laboratory control samples and LOQ Check Standards prepared with verified and known amounts of all target analytes in the sample matrix (e.g. deionized water, sand, commercially available tissue) and by calculating percent recovery. Results are compared against measurement performance specifications and used during evaluation of analytical performance. Program-defined measurement performance specifications for bias are specified in Tables A7.1 and A7.2.

Representativeness

Data collected under this project will be considered representative of ambient water quality conditions. Representativeness is a measure of how accurately a monitoring program reflects the actual water quality conditions typical of a receiving waterbody. The representativeness of the data is dependent on 1) the sampling locations, 2) the number of samples collected, 3) the number of years and seasons when sampling is performed, 4) the number of depths sampled, and 5) the sampling procedures. Site selection procedures will assure that the measurement data represent the conditions at the site. The goal for meeting total representation of the waterbody and watershed is tempered by the availability of time, site accessibility, and funding. Representativeness will be measured with the completion of sample collection in accordance with the approved QAPP.

Comparability

The comparability of the data produced is predetermined by the commitment of the staff to use only approved QA/QC procedures as described in this QAPP. Comparability is also guaranteed by reporting all ambient, high flow and QC data for evaluation by others by reporting data in standard units.

Completeness

The completeness of the data is basically a relationship of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to accidents, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 90% data completion is achieved.

Section A8: Special Training Requirements/Certification

Surface Water Quality Monitoring

All personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. UTB personnel involved in this project have been trained in the appropriate use of field equipment, laboratory equipment, laboratory safety, and all applicable SOPs. Dr. Jude A. Benavides earned a PhD in environmental sciences and engineering with a focus on hydrology, flood alert system design, and hydrologic modeling. He has over 12 years of hydrologic modeling experience that includes using field-based measurements of rainfall, streamflow, and land cover for model calibration. He is currently an Associate Professor of Hydrology and Environmental Sciences at UTB. He also has several years of experience with Geographic Information Systems applications in the field of floodplain mapping and modeling. Dr. Benavides has been involved with research and policy formulation for the Arroyo Colorado basin for over 6 years and has served as the chairperson of the Arroyo Colorado Watershed Partnership's Steering Committee. UTB student researchers employed for this project will be senior students in the Environmental Science program at UTB – an undergraduate program with a strong emphasis on field methods and environmental data collection. Several students in the program have worked on the Arroyo Colorado for current and previously funded research projects.

BST Analysis

All personnel involved in sample analyses and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. Dr. George D. Di Giovanni (UTSPH) earned a PhD in microbiology and immunology with an emphasis in environmental microbiology. He has over 25 years of environmental microbiology experience and is currently Professor of Environmental and Occupational Health Sciences with UTSPH. He has also served as a National Research Council Associate with USEPA, Senior Environmental Scientist for the American Water Works Company, and Professor and Faculty Fellow with the Texas A&M System. His research program focuses on the detection and molecular analysis of waterborne pathogens and microbial source tracking to determine the sources of water fecal pollution. He and his research team have been performing BST analyses for Texas regulatory agencies for over 10 years. UTSPH-EP personnel involved in this project have been trained in the appropriate use of laboratory equipment, laboratory safety, cryogenics safety, and all applicable SOPs. Each laboratory analyst must demonstrate their capability to conduct each test that the analyst performs to the Lab Director. This demonstration of capability is performed before analyzing samples and annually thereafter. Finally, B-PUB has NELAP accreditation for enumerating *E. coli* and *Enterococcus* in both non-potable and drinking water using USEPA Method 1603 and IDEXX Enterolert (NELAP Code#60030208).

Section A9: Documentation and Records

Surface Water Quality Monitoring

Hard copies of general maintenance records, all field data sheets, Chain-Of-Custody Forms (COCs), laboratory data entry sheets, calibration logs, and corrective action reports (CARs) will be archived by each laboratory for at least five years. In addition, UTB and B-PUB will archive electronic forms of all project data for at least five years. All electronic data are backed up on an external hard drive monthly, compact disks weekly, and is simultaneously saved in an external network folder and the computer's hard drive. A blank CAR form is presented in Appendix A, a blank COC form is presented in Appendix C, and blank field data reporting forms are presented in Appendix B.

BST Analysis

Individual laboratory notebooks, which contain printouts of laboratory data and hand written observations and data, are kept by individual analysts at UTSPH-EP or the UTSPH-EP Project Co-Lead for at least five years. When lab notebooks are filled, they are stored for at least five years by the UTSPH-EP Project Co-Lead/Laboratory Manager in hardcopy form. The UTSPH-EP keeps electronic data on personal computers for the duration of the project and then in hardcopy files for 5 years after the project. COCs and attached documents are stored in numerical order in three-ring binders in the UTSPH-EP Project Co-Lead/Laboratory Manager's office for at least five years. In addition, the UTSPH-EP Project Co-Lead/Laboratory Manager will archive electronic forms of all project data for at least five years on personal computers and fire-resistant cabinets. Lab data reports from the UTSPH-EP, as included in the final report, and other reports as required, will report test results clearly and accurately.

Combined Project Documentation

The flow of data will begin with UTB collecting water samples and field parameters and delivering them to B-PUB. Field parameter data will be maintained in a formatted spreadsheet to be shared with B-PUB no less than quarterly or as needed. B-PUB, after enumeration of water samples has been completed, will provide UTB with data in a formatted spreadsheet on a quarterly basis, who will then transfer to TWRI to upload into SWQMIS. Samples plated using EPA 1603 method will be shipped to UTSPH-EP for BST analysis. BST results will be sent to TWRI electronically for inclusion in quarterly progress reports and the final report as appropriate.

Quarterly progress reports disseminated to the individuals listed in section A3 will note activities conducted in connection with the project, items or areas identified as potential problems, and any variations or supplements to the QAPP.

CARs will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference at TWRI and will be disseminated to the individuals listed in section A3.

CARs resulting in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in updates or amendments to the QAPP.

All electronic data are backed up on an external hard drive monthly, compact disks weekly, and is simultaneously saved in an external network folder and the computer's hard drive. A blank CAR is presented in Appendix A and a blank COC form is presented in Appendix C.

The TSSWCB may elect to take possession of records at the conclusion of the specified retention period. Table A9.1 highlights all of the project documents and records along with their specified retention location, time and form.

A final report will be developed upon completion of the project and submitted to TSSWCB that contains all relevant project information and results. The report will be kept in electronic and paper format for no less than 5 years.

Table A9.1. Project Documents and Records

Document / Record	Location	Retention	Form
QAPP, amendments and appendices	TWRI	5 years	Paper/Electronic
Chain of Custody Records	UTB, B-PUB, UTSPH-EP	5 years	Paper
Correction Action Reports	UTB, UTSPH-EP, TWRI	5 Years	Paper
Field notebooks & data sheets	UTB	5 years	Paper
Corrective Action Report	TWRI	5 years	Paper
Bacteriological data logs sheet	B-PUB, UTSPH-EP	5 years	Paper
Laboratory QA Manuals	B-PUB, UTSPH-EP	5 years	Paper
Laboratory SOPs	B-PUB, UTSPH-EP	5 years	Paper
Lab equipment calibration records & maintenance logs	B-PUB, UTSPH-EP	5 years	Paper
Lab data reports	TWRI/TSSWCB	5 years	Paper/Electronic
Quarterly progress reports/final report/data	TWRI/TSSWCB	5 years	Paper/Electronic
Final Report	TWRI/TSSWCB	5 years	Paper/Electronic

QAPP Revision and Amendments

Until the work described is completed, this QAPP shall be revised as necessary and reissued annually on the anniversary date, or revised and reissued within 120 days of significant changes, whichever is sooner. The last approved versions of QAPPs shall remain in effect until revised versions have been fully approved; the revision must be submitted to the TSSWCB for approval before the last approved version has expired. If the entire QAPP is current, valid, and accurately reflects the project goals and the organization's policy, the annual re-issuance may be done by a

certification that the plan is current. This can be accomplished by submitting a cover letter stating the status of the QAPP and a copy of new, signed approval pages for the QAPP.

Amendments to the QAPP may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and non-conformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances. Requests or amendments are directed from the TWRI PM to the TSSWCB PM in writing. The changes are effective immediately upon approval by the TSSWCB PM and QAO, or their designees, and the EPA Project Officer. Amendments to the QAPP and the reasons for the changes will be documented, and copies of the approved QAPP Amendment will be distributed to all individuals on the QAPP distribution list by the TWRI QAO. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

Section B1: Sampling Process Design (Experimental Design)

Surface Water Quality Monitoring

Data collection and analysis will play a pivotal role in this project and will provide data to inform SWCDs and landowners of any potential or existing water quality issues and/or problems and provide information for updating the Arroyo Colorado Watershed Protection Plan. In addition, water samples will be analyzed to determine the source of bacteria entering the stream. This information will be instrumental in evaluating potential BMPs to implement in the watershed as well as aid in the WPP update. Achievement of these objectives will support decisions on how to best target management measures to reduce fecal bacteria levels in the Arroyo Colorado watershed. The constituents that will be measured are shown in Table A7.1.

The sampling program is designed to characterize water quality in the Arroyo Colorado. Water quality grab samples will be collected on monthly intervals for all constituents. Routine grab samples will only be taken if water is flowing at sampling sites. Sampling locations are described in Table A6.1. Physical parameters that will be measured *in situ* during routine sampling include flow (cfs), specific conductance, DO, pH, salinity, and water temperature; other noted items will include the flow severity, days since last significant rainfall, water depth, and present weather conditions. Sites that are dry or with pooled water will be noted on the field data sheet and not sampled. Water quality samples collected as part of the routine sampling schedule will be analyzed for *E. coli* and Enterococcus as outlined in Table A7.2. Field data and samples will be collected following procedures detailed in the most recent version of the *TCEQ SWQM Procedures, Volume 1 (RG-415).* Additional water samples and field blanks will be collected and delivered to B-PUB for *E. coli* analysis and preparation for future BST analysis.

In order to obtain representative results, ambient water sampling will occur on a routine schedule over the course of 12 months. There will be no prejudice against rainfall or high flow events, except that the safety of the sampling crew will not be compromised in case of lightning or flooding; this is left up to the discretion of the sampling crew.

In the instance that a sampling (Table A6.1) site is inaccessible, no sample will be taken and will be documented in the field notebook. If, near the end of the study, the TSSWCB PM/QAO agrees that the sampling has not achieved good representativeness of typical conditions, the final sampling event(s) may be restricted to target a particular environmental condition (e.g., rainfall).

BST Analysis

Water samples collected by UTB will be delivered to the B-PUB for *E. coli* enumeration using EPA Method 1603, with the plates saved and shipped to UTSPH-EP for BST analysis. UTSPH-EP will isolate and fingerprint (ERIC-RP) *E. coli* (five water *E. coli* per site per sample event) for 12 sampling events at the 10 stream sites. This results in a total of 600 *E. coli* isolates from 120 individual samples analyzed using ERIC-PCR and RiboPrinting.

Fecal material samples from known sources also will be collected and used to expand the Texas *E. coli* BST Library for identifying sources of the water isolates. Approximately 200 known source samples from different individual animals will be collected throughout the course of the project and will include domestic animals, wildlife and human sources. These known sources of bacteria (domestic animals, wildlife, and humans) will not be collected from the same locations during every collection due to the nature of the animals. Sewage and septage samples (not from individuals) will represent human sources and will be collected based on granted access to facilities.

Section B2: Sampling Method Requirements

Surface Water Quality Monitoring

Field Sampling Procedures
Field sampling will be conducted according to procedures documented in the most recent version of the *TCEQ Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue (RG-415)*. Additional aspects outlined in Section B below reflect specific requirements for sampling.

Field sampling activities are documented on field data reporting forms as presented in Appendix B. The following will be recorded for all water sampling:

- station ID
- location
- sampling time
- date
- water depth
- flow rate
- sample collector's name/signature

Detailed observational data are recorded including water appearance, weather, biological activity, stream uses, unusual odors, specific sample information, days since last significant rainfall, estimated hours since rainfall began (if applicable), and flow severity.

Typically, water samples will be collected directly from the stream (midway in the stream channel) into sterile wide-mouthed polypropylene bottles or bags. Water samples used for *E. coli* and Enterococcus analysis will be collected in a 500 ml sterile polyethylene bottle provided by UTB. All sample containers will be labeled with the following information:

- collection date
- collection time
- sample location
- sampler's initials

Care will be exercised to avoid the surface microlayer of water, which may be enriched with bacteria and not representative of the water column. In cases where, for safety reasons, it is inadvisable to enter the stream bed, and boat access is not practical, staff will use a clean bucket and rope from a bridge to collect the samples from the stream. If a bucket is used, care will be taken to avoid contaminating the sample. Specifically, technicians must exert care to ensure that the bucket and rope do not come into contact with the bridge. The bucket must be thoroughly rinsed between stations. Buckets are also to be sanitized between sampling stations with a bleach- or isopropyl alcohol-soaked wipe. The first bucketful of water collected from a bridge is

used to rinse the bucket. Rinse water is not returned to the stream, but is instead disposed of away from the sampling site to ensure that the collected sample will not be affected by the bleach or alcohol residual. Samples are collected from subsequent buckets of water. This type of sampling will be noted in the field records.

Water temperature, stream flow, pH, specific conductivity, specific conductance, and DO will be measured and recorded *in situ* with a multiprobe whenever samples are collected. All field measurements will be conducted in accordance with the methods listed in Table B4.1. Measurements will only be taken if water is flowing. If a site is not flowing but pooled or dry, that will be noted on the field data sheet. All samples will be transported in an iced container to the laboratory for analysis.

Table B2.1. Storage, Preservation and Handling Requirements

Parameter	Matrix	Container	Preservation	Temp	Sample Size	Holding Time
E. coli	Water	Sterile container	n/a	4°C	250 ml	6 hours for enumeration, modified mTEC plates 72 hours after enumeration for delivery to UTSPH-EP
Fecal Specimen	Feces	Sterile container	n/a	4°C	30g	72 hrs until plating on modified mTEC
E. coli isolates from water and fecal samples	modified mTEC agar	Petri dish 100 mm x 15 mm	n/a	4°C	5-8 colony streaks	72 hrs, until plating on NA-MUG
E. coli isolates from water and fecal samples	NA-MUG	Petri dish 100 mm x 15 mm	n/a	4°C	1 colony each	24 hrs. until archival
E. coli isolates from water and fecal samples	20% glycerol; 80% tryptic soy broth	Archive tube	n/a	-80°C	1 colony each	frozen indefinitely
Enterococcus	Water	Sterile PE	n/a	4°C	250 ml	6 hours

Fecal Sampling Method Requirements

To ensure fresh samples of known origin, fecal samples will be obtained using one of five methods: a) collected from animals visually observed defecating by technician; b) collected from cages of trapped animals; c) collected from intestines of animals recently killed by cars (within 24 hours); d) collected from intestines of animals legally harvested; or e) human (wastewater) samples collected from individual septic tanks, composite septic samples from pump trucks, wastewater treatment plant influent (for plants with secondary disinfection or lagoon treatment), or from lagoon treatment effluents. All fecal samples will be shipped to UTSPH-EP for BST analysis within 3 days of collection. Only fecal material will be shipped to UTSPH-EP.

If trapping is required, project personnel will randomly locate trap arrays on properties where permission has been received in order to capture species that contribute to the bacteria impairment. Animals will be released safely and once clear of the area, technicians will collect feces. After releasing animals from the trap and collecting fecal samples, the cage will be cleaned and moved to prevent possible cross contamination of subsequent fecal samples. Traps will be closed every morning and reopened every evening during each trap session to prevent animals from being confined in cages in daylight hours. Traps will be set in shaded areas to reduce heat stress on the animals and for their safety. During periods of high temperature, trapping may be rescheduled.

Documentation of Field Sampling Activities

Each fecal sample will be collected in a sterile fecal tube (Sarstedt, cat# 80.734.311). Wastewater samples can initially be collected with sterile bottles or other suitable device and then transferred to the fecal tubes. Specimen container tubes will be labeled with:

- Sampling date
- Sampling time
- Animal species
- Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
- Sample collector's name
- Sampler's initials
- Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle

Samples should be refrigerated (~4°C) or kept on ice following collection and shipped to UTSPH-EP on ice within 3 days of collection. See SOP in Appendix D-2 for complete protocol.

Safety is an issue when working with fecal samples due to the bacterial concentration. Hazardous material safety handling instructions will be included in a file for the driver to carry that will be visible on the seat or dash of the vehicle in case of accident or being stopped by law enforcement officers. Biohazard signs will be placed on the cooler containing samples collected for transport. Sheets of Lading (Appendix C-2) will be on hand with the field technician and completed for each fecal sample collected along with a COC form (Appendix C).

Failures in Sampling Methods Requirements and/or Deviations from Sample Design and Corrective Action

Examples of failures in sampling methods and/or deviations from sample design requirements include, but are not limited to, such things as sample container problems, sample site considerations, etc. Failures or deviations from the QAPP are documented on the field data reporting form and reported to the UTB Project Leader. The UTB Project Leader will determine if the deviation from the QAPP compromises the validity of the resulting data. The UTB Project Leader, in consultation with the TWRI QAO and TSSWCB QAO will decide to accept or reject data associated with the sampling event, based on best professional judgment. The resolution of the situation will be reported to the TSSWCB in the QPR and by completion of a CAR. The CAR documents: root cause(s); programmatic impact(s); specific corrective action(s) to address the deficiency; action(s) prevent recurrence; individual(s) responsible for each action; the timetable for completion of each action; and, the means by which completion of each corrective action will be documented (see Appendix A). In addition, significant conditions (i.e., situations that, if uncorrected, could have a serious effect on safety or validity or integrity of data) will be reported to the TSSWCB immediately both verbally and in writing.

Section B3: Sample Handling and Custody Requirements

Surface Water Quality Monitoring

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. The COC form is used to document sample handling during transfer from the field to the laboratory and inter-laboratory. The sample collector will sign the COC and transport it with the sample to the laboratory. At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. Sample information will be entered into B-PUB - Analytical Laboratory Information Management System (LIMS) upon receipt of the samples. The LIMS will generate a unique sample identification number for the sample, which will be affixed to each container. A sample receipt log will be printed each day and maintained on file. The following information concerning the sample is recorded in indelible ink on the COC form (See Appendix C):

1. Date and time of collection
2. Site identification
3. Sample matrix
4. Number of containers
5. Preservative used
6. Was the sample filtered
7. Analyses required
8. Name of collector
9. Custody transfer signatures and dates and time of transfer
10. Bill of lading (if applicable)

Sample Labeling

Samples will be labeled on the container with an indelible, waterproof marker. Label information includes:

- Site identification
- Date and time of sampling
- Preservative added, if applicable
- Designation of “field-filtered” (for metals) as applicable
- Sample type (i.e., analysis(es)) to be performed

The COC form will accompany all sets of sample containers.

Sample Handling

Following collection, samples will be placed on ice in an insulated cooler for transport to the laboratory. At the laboratory, samples will be placed in a refrigerated cooler dedicated to sample storage. The Laboratory Supervisor has the responsibility to ensure that holding times

are met with water and fecal samples. The collection and deliver time is documented on the COC. Any problem will be documented with a CAR.

Failures in Chain-of-Custody and Corrective Action

All failures associated with COC procedures are to be immediately reported to the TWRI PM. Failures include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The TSSWCB PM and QAO, in consultation with the TWRI PM and QAO, will determine if the procedural violation may have compromised the validity of the resulting data. Any failure that potentially compromises data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB PM in a CAR and submitted to the TSSWCB PM along with the QPR. The CARs will be maintained by the TWRI PM.

BST Analysis

The same sample handling and custody procedures followed under the *Surface Water Quality Monitoring* section above apply here.

Section B4: Analytical Methods

Surface Water Quality Monitoring

The analytical methods, associated matrices, and performing laboratories are listed in Table A7.1 of Section A7. Procedures for laboratory analysis will be conducted in accordance with the most recently published edition of *Standard Methods for the Examination of Water and Wastewater*, the latest version of the *SWQM Procedures, Volume 1: Physical Methods for Water, Sediment, and Tissue*, 40 CFR 136, or approved *EPA Methods for Chemical Analysis of Water and Wastes.*

Laboratories collecting and analyzing data under this QAPP are compliant with the NELAP standards where required. Copies of laboratory QMs and SOPs are available for review by the TSSWCB.

Standards Traceability
All standards used in the field and laboratory are traceable to certified reference materials. Standards preparation is fully documented and maintained in a standards log book. Each documentation includes information concerning the standard identification, starting materials, including concentration, amount used and lot number; date prepared, expiration date and preparer's initials/signature. The reagent bottle is labeled in a way that will trace the reagent back to preparation.

Analytical Method Deficiencies and Corrective Actions
Deficiencies in field and laboratory measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, quality control samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the B-PUB – Analytical Laboratory / UTSPH-EP Laboratory Supervisor, who will make the determination and notify the B-PUB – Analytical Laboratory. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TCEQ, but will be submitted on the data report sent to the TSSWCB PM. The nature and disposition of the problem is reported on the data report which is sent to the TWRI PM. This information will be included in the CAR and submitted with the QPR, which is sent to the TSSWCB PM.

The definition of and process for handling deficiencies and corrective action are defined in Section C1.

BST Analysis

The analytical methods utilized in BST analysis and sample preparation are listed in Table B4.1 and described in detail in Appendix D.

E. coli in water samples will be isolated and quantified by B-PUB - Analytical Laboratory personnel using modified mTEC agar, EPA Method 1603 [EPA/821/R-02/023. December 2009. *Escherichia coli* in Water by Membrane Filtration Using modified Membrane-Thermotolerant *Escherichia coli* (modified m-TEC) Agar]. The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with 4-methylumbelliferyl-β-D-glucuronide (MUG) observed by ultraviolet light (UV) fluorescence. These Method 1603 plates will be shipped to UTSPH-EP for further isolation and analysis.

Fecal specimens or domestic sewage samples collected by TWRI or project partners and sent to UTSPH-EP will be streaked (resuspended in buffer if necessary) onto modified mTEC medium. The use of modified mTEC medium for isolation of *E. coli* from both water and source samples will help avoid selection of different types of *E. coli* due to different media. Inoculated plates will be incubated at 35±0.5^{o}C for 2 hours to resuscitate stressed bacteria and then incubated at 44.5±0.2^{o}C for approximately 20 to 24 hours.

E. coli colonies from the modified mTEC medium will be picked and streaked for purity on nutrient agar with MUG (NA-MUG) to confirm glucuronidase activity and culture purity for both water and source sample isolates. Cultures of selected isolates will be archived using glycerol freezing medium (-80^{o}C). Up to 8 isolates per site per sampling event will be archived from water samples, and up to 5 isolates per sample will be archived for fecal samples.

Up to 5 *E. coli* isolates from each water sample will be fingerprinted using ERIC-RP. Up to 3 isolates per fecal sample will be screened for clones using ERIC-PCR. Non-clonal isolates will then be RiboPrinted and included in the local watershed library. After screening the ERIC-RP fingerprints of these known source samples for host specificity, they will be included in the Texas *E. coli* BST Library of *E. coli* isolates from known animal and human sources collected throughout Texas. *E. coli* isolates obtained from the ambient water samples from across the study area will also be characterized using ERIC-PCR and RiboPrinting using UTSPH-EP SOPs. The composite DNA patterns of those isolates will be compared to the Texas *E. coli* BST Library (which will include the isolates from the local known source samples).Water isolates will be identified to cattle, other livestock, avian wildlife, non-avian wildlife, domestic sewage, and pet sources (six-way split), as well as a general three-way split of livestock, domestic sewage and wildlife using a best match approach and an 80% similarity cutoff.

Enterococcus in water samples will be quantified by IDEXX/ASTM D6503-99, Enterococcus NELAP Code #60030208. (Standard Method SM9223 - Chromogenic Substrate Test includes total coliforms and *E. coli*, but not *Enterococcus*.

All laboratory sampling areas and equipment will be sterilized with at least one or in any combination of the following methods: ethyl alcohol, bleach, UV light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal.

Failures in Measurement Systems and Corrective Actions

Failures in field and laboratory measurement systems involve, but are not limited to, such things as instrument malfunctions, failures in calibration, blank contamination, QC samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the UTSPH-EP Project Co-Lead, who will make the determination in coordination with the TWRI PM/QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB as part of this project. The nature and disposition of the problem is reported on the data report. The TWRI PM/QAO will include this information in the CAR and submit with the QPR which is sent to the TSSWCB PM.

Table B4.1. Laboratory Analytical Methods

Parameter	Method	Equipment Used
Laboratory Parameters		
E. coli	EPA 1603	Filtration apparatus, incubator
E. coli ERIC-PCR fingerprint	UTSPH-EP SOP	PCR thermal cycler, gel electrophoresis apparatus
E. coli RiboPrint fingerprint	UTSPH-EP SOP	RiboPrinter
Enterococcus	IDEXX Enterolert,NELAP Code #60030208	IDEXX Sealer
Field Parameters		
pH	EPA 150.1 & TCEQ SOP, V1	
DO	SM 4500-O G & TCEQ SOP, V1	
Specific Conductance	EPA 120.1 & TCEQ SOP, V1	
Days since last significant rainfall	TCEQ SOP, V1	Field Observation
Flow	TCEQ SOP, V1	
Total Water Depth	TCEQ SOP, V1	Wading Rod
Flow Measurement Method	TCEQ SOP, V1	
Flow Severity	TCEQ SOP, V1	Field Observation
Present Weather	TCEQ SOP, V1	Field Observation

EPA = Methods for Chemical Analysis of Water and Wastes, March 1983
SM = Standard Methods for Examination of Water and Wastewater, 21st edition
SOP = Standard Operating Procedure

Section B5: Quality Control Requirements

Surface Water Quality Monitoring

Sampling Quality Control Requirements and Acceptability Criteria
The minimum Field QC Requirements are outlined in the *TCEQ Surface Water Quality Monitoring Procedures.* Specific requirements are outlined below. Field QC sample results are submitted with the laboratory data report (see Section A9).

Field Split
A field split is a single sample subdivided by field staff immediately following collection and submitted to the laboratory as two separately identified samples according to procedures specified in the *SWQM Procedures*. Split samples are preserved, handled, shipped, and analyzed identically and are used to assess variability in all of these processes. Field splits apply to conventional samples only and are collected on a 10% basis. If less than ten samples are collected in a month, one set of field splits will be collected per month. The precision of field split results is calculated by relative percent difference (RPD) using the following equation:

$$RPD = |(X1 - X2)/\{(X1+X2)/2\} * 100|$$

A 30% RPD criteria will be used to screen field split results as a possible indicator of excessive variability in the sample handling and analytical system. If it is determined that elevated quantities of analyte (e.g., > 5 times the LOQ) were measured and analytical variability can be eliminated as a factor, than variability in field split results will primarily be used as a trigger for discussion with field staff to ensure samples are being handled in the field correctly. Some individual sample results may be invalidated based on the examination of all extenuating information. The information derived from field splits is generally considered to be event specific and would not normally be used to determine the validity of an entire batch; however, some batches of samples may be invalidated depending on the situation. Professional judgment during data validation will be relied upon to interpret the results and take appropriate action. The qualification (e.g., invalidation) of data will be documented on the data summary. Deficiencies will be addressed as specified in this section under Quality Control or Acceptability Requirements Deficiencies and Corrective Actions.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

Batch
A batch is defined as environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of up to 20 environmental samples of the same NELAP-defined matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 25 hours. An **analytical batch** is composed of prepared environmental samples (extract, digestates or concentrates) which are analyzed together as a

group. An analytical batch can include prepared samples originating from various environmental matrices and can exceed 20 samples.

Method Specific QC requirements
QC samples, other than those specified later this section, are run (e.g., sample duplicates, surrogates, internal standards, continuing calibration samples, interference check samples, positive control, negative control, and media blank) as specified in the methods. The requirements for these samples, their acceptance criteria or instructions for establishing criteria, and corrective actions are method-specific.

Detailed laboratory QC requirements and corrective action procedures are contained within the individual laboratory quality manuals (QMs). The minimum requirements that all participants abide by are stated below.

Limit of Quantitation (LOQ)
The laboratory will analyze a calibration standard (if applicable) at the LOQ on each day calibrations are performed. In addition, a LOQ check standard will be analyzed with each analytical batch. Calibrations including the standard at the LOQ will meet the calibration requirements of the analytical method or corrective action will be implemented.

LOQ Check Standard
An LOQ check standard consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system at the lower limits of analysis. The LOQ check standard is spiked into the sample matrix at a level less than or near the LOQ for each analyte for each analytical batch of samples run.

The LOQ check standard is carried through the complete preparation and analytical process. LOQ Check Standards are run at a rate of one per analytical batch. The percent recovery of the LOQ check standard is calculated using the following equation in which %R is percent recovery, SR is the sample result, and SA is the reference concentration for the check standard:

$$\%R = SR/SA * 100$$

Measurement performance specifications are used to determine the acceptability of LOQ Check Standard analyses as specified in Table A7.1.

Laboratory Control Sample (LCS)
An LCS consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system. The LCS is spiked into the sample matrix at a level less than or near the mid-point of the calibration for each analyte.

In cases of test methods with very long lists of analytes, LCSs are prepared with all the target analytes and not just a representative number, except in cases of organic analytes with multi-peak responses.

The LCS is carried through the complete preparation and analytical process. LCSs are run at a rate of one per preparation batch. Results of LCSs are calculated by percent recovery (%R), which is defined as 100 times the measured concentration, divided by the true concentration of the spiked sample. The following formula is used to calculate percent recovery, where %R is percent recovery; SR is the measured result; and SA is the true result:

$$\%R = SR/SA * 100$$

Measurement performance specifications are used to determine the acceptability of LCS analyses as specified in Table A7.1.

Laboratory Duplicates

A laboratory duplicate is prepared by taking aliquots of a sample from the same container under laboratory conditions and processed and analyzed independently. A laboratory control sample duplicate (LCSD) is prepared in the laboratory by splitting aliquots of an LCS. Both samples are carried through the entire preparation and analytical process. LCSDs are used to assess precision and are performed at a rate of one per preparation batch.

For most parameters, precision is calculated by the relative percent difference (RPD) of LCS duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X1 and X2, the RPD is calculated from the following equation:

$$RPD = |(X1 - X2)/\{(X1+X2)/2\} * 100|$$

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the lab. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair. Measurement performance specifications are used to determine the acceptability of duplicate analyses as specified in Table A7.1. The specifications for bacteriological duplicates in Table A7.1 apply to samples with concentrations > 10 organisms/100 ml.

Matrix spike (MS)

Matrix spikes are prepared by adding a known mass of target analyte to a specified amount of matrix sample for which an independent estimate of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method’s recovery efficiency.

Percent recovery of the known concentration of added analyte is used to assess accuracy of the analytical process. The spiking occurs prior to sample preparation and analysis. Spiked samples are routinely prepared and analyzed at a rate of 10% of samples processed, or one per preparation batch whichever is greater. The information from these controls is sample/matrix specific and is not used to determine the validity of the entire batch. To the extent possible, matrix spikes prepared and analyzed over the course of the project should be performed on samples from different sites. The MS is spiked at a level less than or equal to the midpoint of the calibration or analysis range for each analyte. Percent recovery (%R) is defined as 100 times the observed concentration, minus the sample concentration, divided by the true concentration of the spike.

The results from matrix spikes are primarily designed to assess the validity of analytical results in a given matrix and are expressed as percent recovery (%R). The laboratory shall document the calculation for %R. The percent recovery of the matrix spike is calculated using the following equation in which %R is percent recovery, SSR is the observed spiked sample concentration, SR is the sample result, and SA is the reference concentration of the spike added:

$$\%R = (SSR - SR)/SA * 100$$

The results are compared to the acceptance criteria as published in the mandated test method. Where there are no established criteria, the laboratory shall determine the internal criteria and document the method used to establish the limits. The laboratory has established limits for matrix spike recovery of 80-120% unless more stringent limits are mandated by the method. For matrix spike results outside established criteria, corrective action shall be documented or the data reported with appropriate data qualifying codes.

Method blank

A method blank is a sample of matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. The method blanks are performed at a rate of once per preparation batch. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the LOQ. For very high-level analyses, the blank value should be less than 5% of the lowest value of the batch, or corrective action will be implemented. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the samples (e.g. reprocessing or data qualifying codes). In all cases the corrective action must be documented.

The method blank shall be analyzed at a minimum of one per preparation batch. In those instances for which no separate preparation method is used (example: volatiles in water) the batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.

Quality Control or Acceptability Requirements Deficiencies and Corrective Actions
Sampling QC excursions are evaluated by the UTB manager in consultation with the UTSPH-EP Co-Project Lead. In that differences in sample results are used to assess the entire sampling process, including environmental variability, the arbitrary rejection of results based on pre-determined limits is not practical. Therefore, the professional judgment of the UTB and UTSPH-EP Co-Project Leads will be relied upon in evaluating results. Rejecting sample results based on wide variability is a possibility. Field blank values exceeding the acceptability criteria may automatically invalidate the sample, especially in cases where high blank values may be indicative of contamination which may be causal in putting a value above the standard. Notations of field split excursions and blank contamination are noted in the quarterly report and the final QC Report.

Laboratory measurement quality control failures are evaluated by the laboratory staff. The disposition of such failures and the nature and disposition of the problem is reported to the TWRI PM and QAO. If applicable, the UTSPH-EP Project Co-Lead will include this information in the CAR and submit with the QPR, which is sent to the TSSWCB PM.

The definition of and process for handling deficiencies and corrective action are defined in Section C1.

BST Analysis

Table A7.1 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the UTSPH-EP Project Co-Lead to verify that the data are representative. The UTSPH-EP Project Co-Lead also has the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of a CAR (Appendix A).

Laboratory Blanks
Laboratory blanks, or negative controls, consist of 100-ml aliquots of sterile distilled water that are processed in the same manner as a field sample, at the beginning and the end of a sample set. They are used to assess the sterilization techniques employed throughout the sample process. Laboratory blanks will be included at the beginning and the end of the sample set for each sampling event. The analysis of laboratory blanks should yield a value of no colonies detected. No template negative controls will be analyzed for each batch of ERIC-PCR and will serve as its method blank.

Positive Control
Positive controls will be analyzed for each batch of *E. coli* ERIC-PCR and RiboPrinting using a laboratory control *E. coli* strain.

Laboratory Duplicate
Laboratory duplicates are used to assess precision. A laboratory duplicate is prepared by splitting aliquots of a single sample (or a matrix spike or a laboratory control standard) in the

laboratory. Both samples are carried through the entire preparation and analytical process. Laboratory duplicates are run at a rate of one per batch. Acceptability criteria are outlined in Table A7.1 of Section A7.

Precision is calculated by the relative percent difference (RPD) of duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, X_1 and X_2, the RPD is calculated from the following equation:

$$\mathbf{RPD} = \frac{(X_1 - X_2) \times 100}{(X_1 + X_2) \div 2}$$

A bacteriological duplicate is considered to be a special type of laboratory duplicate and applies when bacteriological samples are run in the field as well as in the laboratory. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair.

Performance limits and control charts are used to determine the acceptability of duplicate analyses. Precision limits for bacteriological analyses are defined in Table A7.1 and applies to samples with concentrations >10 CFU/100 ml.

Failures in Quality Control and Corrective Action

Notations of blank contamination will be noted in QPRs and the final report. Corrective action will involve identification of the possible cause (where possible) of the contamination failure. Any failure that has potential to compromise data validity will invalidate data, and the sampling event should be repeated. The TWRI PM/QAO will include this information in the CAR and submit with the QPR which is sent to the TSSWCB PM.

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

Surface Water Quality Monitoring

All sampling equipment testing and maintenance requirements are detailed in the most recent version of the *TCEQ Surface Water Quality Monitoring Procedures, Volume 1*. Sampling equipment is inspected and tested upon receipt and is assured appropriate for use. Equipment records are kept on all field equipment and a supply of critical spare parts is maintained. All laboratory tools, gauges, instrument, and equipment testing and maintenance requirements are contained within the laboratory QM(s).

BST Analysis

To minimize downtime of all measurement systems, spare parts for laboratory equipment will be kept in the laboratory, and all laboratory equipment must be maintained in a working condition. All laboratory equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in *Standard Methods for the Examination of Water and Wastewater, 21st Edition*. Maintenance and inspection logs will be kept on each piece of laboratory equipment. Table B6.1. identifies the equipment and maintenance requirements for both surface water quality monitoring and BST analysis below.

Records of all tests, inspections, and maintenance will be maintained and log sheets kept showing time, date, and analyst signature. These records will be available for inspection by the TSSWCB.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. The CARs will be maintained by the UTSPH-EP.

Table B6.1. Equipment Inspection and Maintenance Requirements

4. Laboratory Equipment and Instrumentation	Relevant Testing, Inspection & Maintenance Requirements
Thermometers	SM 9020 B 4.a
PCR Thermal cycler	Per manufacturer
RiboPrinter	Per manufacturer & annual preventative maintenance
Balances	SM9020 B 4.b
pH Meter	SM 9020 B 4.c
Water deionization units	SM 9020 B 4.d
Multi-well trays and sealers	SM9020 B 4.e
Media dispensing apparatus	SM 9020 B 4.f
Hot-air Sterilizing Oven	SM 9020 B 4.g
Autoclaves	SM 9020 B 4.h
Refrigerator	SM 9020 B 4.i
Ultra Low Freezer	SM 9020 B 4.j

Membrane filter equipment	SM 9020 B 4.k
Ultraviolet sterilization lamps	SM 9020 B 4.l
Biological safety cabinet	SM 9020 B 4.m
Water Bath incubator	SM 9020 B 4.n
Incubators	SM 9020 B 4.o
Conductivity Meter	SM 9020 B 4.q
Micropipettors	SM 9020 B 4.s
5. Laboratory Supplies	
Glassware and plastic ware	SM 9020 B 5.a
Utensils and containers	SM 9020 B 5.b
Dilution water bottles	SM 9020 B 5.c
Sample Bottles	SM 9020 B 5.d
Multi-well trays and sealers	SM 9020 B 5.e

Section B7: Instrument Calibration and Frequency

Surface Water Quality Monitoring

Field equipment calibration requirements are contained in the most recent version of the *TCEQ Surface Water Quality Monitoring Procedures, Volume 1*. Post-calibration error limits and the disposition resulting from error are adhered to. Data not meeting post-error limit requirements invalidate associated data collected subsequent to the pre-calibration and are not submitted to the TCEQ.

Detailed laboratory calibrations are contained within the QM(s).

BST Analysis

All instruments or devices used in obtaining environmental data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. The instruments requiring calibration are listed below in Table B7.1.

All calibration procedures will meet the requirements specified in the approved methods of analysis. The frequency of calibration as well as specific instructions applicable to the analytical methods recommended by the equipment manufacturer will be followed. All information concerning calibration will be recorded in a calibration logbook by the person performing the calibration and will be accessible for verification during a laboratory audit.

All instruments or devices used in obtaining environmental data will be used according to appropriate laboratory practices. Written copies of SOPs are available for review upon request.

Standards used for instrument or method calibrations shall be of known purity and be National Institute of Standards and Technology (NIST) traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society or reagent grade quality, or of the best attainable grade. All certified standards will be maintained traceable with certificates on file in the laboratory. Dilutions from all standards will be recorded in the standards log book and given unique identification numbers. The date, analyst initials, stock sources with lot number and manufacturer, and how dilutions were prepared will also be recorded in the standards log book.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. The CARs will be maintained by the TWRI PM.

Table B7.1. Instrument Calibration Requirements

Equipment	Relevant Calibration Requirement
RiboPrinter	Per manufacturer & annual preventative maintenance
PCR Thermal Cycler	Per manufacturer

Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

Surface Water Quality Monitoring

All sampling equipment testing and maintenance requirements are detailed in the *TCEQ Surface Water Quality Monitoring Procedures*. Sampling equipment is inspected upon receipt and is assured appropriate for use. All other miscellaneous consumable supplies such as batteries and office supplies are purchased where needed.

All consumable laboratory supplies are purchased from reputable scientific supply dealers.

BST Analysis

All standards, reagents, media, plates, filters, and other consumable supplies are purchased from manufacturers with performance guarantees, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality, initialed by staff member and marked with receipt date. Volumetric glassware is inspected to ensure class "A" classification, where required. Media will be checked as described in QC procedures. All supplies will be stored as per manufacturer labeling and discarded past expiration date. In general, supplies for microbiological analysis are received pre-sterilized, used as received, and not re-used.

Section B9: Data Acquisition Requirements (Non-direct Measurements)

Surface Water Quality Monitoring

Flow at IBWC station 08-4704.00 Arroyo Colorado Floodway South of Harlingen, TX (http://www.ibwc.state.gov/Water_Data/histflo1.htm) is the only non-direct measurement that will occur and data that corresponds with the time of sample collection will be downloaded. This IBWC station corresponds with TCEQ station 13079 Arroyo Colorado at U.S. 77 in Southwest Harlingen.

BST Analysis

Data analyzed using BST analysis methods for this project will consist solely of data produced during the course of this study and will adhere to the guidance set forth in this QAPP. The Texas *E. coli* BST Library is composed of known source fecal *E. coli* isolates obtained under other Texas BST studies with approved QAPPs and will also be supplemented with known source fecal *E. coli* from this study.

Section B10: Data Management

Surface Water Quality Monitoring

All field collection will be completed as described in Section B2 of the QAPP. A COC is filled out for each sampling event noting the site name, time and date of collection, sample type, comments, sample collector's name, and other pertinent data. Samples collected will be labeled with site identification, date, sampler's initials, and time of sampling and transported to the laboratory as outlined in B3. Finally, the COC and accompanying sample bags/bottles are submitted to B-PUB and UTSPH-EP with relinquishing and receiving personnel both signing and dating the COC.

It is imperative that data and associated applications be maintained and managed in a manner consistent with the development and use of the data; in this case, data will be maintained so that they are consistent with project requirements. For scientifically valid results, the data, program applications, and reports must be handled in an orderly and consistent manner. Documented quality assurance and quality control checks/procedures are applied to all received data sets, individual data points and data manipulation programs.

Data will be incorporated into the UTB database and subject to varying levels of review. The QA/QC checks evaluate each data set as a whole, and the validity of individual data points. Each data set to be processed into the database is evaluated for any problems that might impose a limitation on the use of the data. This check is performed prior to processing/importing to the database. The following information is considered:

a. Credibility of data source
b. Acceptable QA/QC procedures
c. Intended use of the data
d. Frequency of data collection/impact of missed sampling events
e. Sample size
f. Sample collection and preservation methods
g. Field and laboratory test procedures
h. General documentation

Upon passing the evaluation of a data set's limitations, the data are incorporated into the UTB database. Initially data are entered, either manually or electronically, into a set of working directory files that are consistent with the UTB database file structures. In the event that a deviation is found in the data set, the corresponding data points will be coded with a "D" in the remarks section of the Results Table. The remark "D" code refers to the SWQMIS data qualifiers, which means 'did not pass all QC criteria. Any deviation found in the data set will be conveyed to the TWRI PM by UTB. Disqualified data will be removed from the dataset and will not be submitted to the TSSWCB for inclusion in SWQMIS. The reason for the data removal will be listed on the data summary report.

Electronic data input procedures vary according to the source and format of the data. Manual data input will be made to appropriately structured MS Access tables. Standardized procedures are followed to ensure proper data entry.

After the data/datasets have been input/converted into an appropriate working directory database, the individual data points will be evaluated to determine their reasonableness. Data values that are considered outliers will be discarded or coded prior to entry into the records directory. The criteria for determination of outliers will be based on individual data sets being processed for entry into the TCEQ's SWQMIS database. Once the dataset is complete, any individual points falling outside the most recent Max/Min range as defined by the TCEQ SWQM Parameters Table will be considered outliers. If an outlier does occur, then it will be noted in the remark section of the database and verified against the original data report, and if necessary, verified by the laboratory. After verification, outliers will either be assigned the appropriate remark code or documented as verified with a 1 in the verify_flg section of the results table.

After the final QA checks are performed by TWRI, data are submitted to the TSSWCB PM. Data are then transferred from the TSSWCB PM to the TCEQ Data Manager, who then loads the data into SWQMIS.

Only data collected under this project and its QAPP will be transferred. The tag series transferred is documented on the Data Summary (QAPP Appendix F) that is submitted to the TCEQ upon the completion of the data transfer. All QA data sets associated with the data transfer will be submitted in the form of a QA Table. The files are transferred as pipe delimited text file format as described in the *Surface Water Quality Monitoring Data Management Reference Guide, 2012* or most recent version to the TSSWCB PM. After data have been transferred, reviewed, and loaded into the TCEQ SWQMIS Database, a link will provided to the TCEQ's Surface Water Quality Web Reporting Tool at http://www80.tceq.texas.gov/SwqmisWeb/public/index.faces for public access.

Data Dictionary - Terminology and field descriptions are included in the SWQM Data Management Reference Guide, 2012 or most recent version. For the purposes of verifying which entity codes are included in this QAPP, the following will be used when submitting data under this QAPP:

Name of Monitoring Entity:	University of Texas at Brownsville
Tag Prefix:	TX
Submitting Entity:	Texas State Soil and Water Conservation Board (TX)
Collecting Entity:	University of Texas at Brownsville (UTB)

Data Errors and Loss

To prevent loss of data and minimize errors, all data generated under this QAPP are verified against the appropriate quality assurance checks as defined in the QAPP, including but not limited to chain of custody procedures, field sampling documentation, laboratory analysis

results, and quality control data. The data are also verified by UTB comparing 10% of the data in the database to hard copy reports as a check against transcription errors.

Backup/Disaster Recovery Requirements
All data associated with UTB's database and network files are completely backed-up daily. See record keeping and data storage section below for more details. The IBM Server PC is protected by an Internet Office UPS with battery backup and surge protection to safely work through blackouts and save open network files.

Should the computer system or software fail, UTB will request the assistance of a Computer/Network Specialist to evaluate the probable cause of the failure, methods to prevent reoccurrence of the problem, and guide recovery of the system. The archived tape backups will allow for complete recovery of the hard disk drive contents.

Record Keeping and Data Storage
A three ring binder will be used as a data set log to track all hard copy data sets associated with the UTB Database. The database management log will also record the structure of tables, data modifications and updates, and record of dates for all file revisions.

Complete original electronic data sets are archived on 40GB backup tapes via an internal tape drive with MS Windows 2000 Server software. Electronic data are backed up on a daily basis Monday through Friday of each work week. The weekly tapes in use are stored at an off-site location to prevent loss due to a disaster such as fire or flood. These tapes are maintained indefinitely until they are replaced by a new set of backup tapes. The original hard copies of field data sheets and laboratory reports are stored in binders at the UTB offices for a minimum period of five years.

Data Handling, Hardware, and Software Requirements
UTB has put into place an electronic data processing system consisting of a network with the following configuration:

System Design
UTB utilizes standard, IBM compatible, desktop personal computers that utilize the MS Windows XP operating system. Software operated includes MS Office Pro, Corel WordPerfect Office 2000, Accounting Express 2008 and Front Page 2002.

UTB utilizes MS Access 2007 as the primary database management software. ANRA's Water Quality Database has been developed according to CRP guidance and database structures in accordance with TCEQ requirements.

Information Resource Management Requirements
Data will be managed in accordance with the TCEQ Surface Water Quality Monitoring Data Management Reference Guide and applicable Basin Planning Agency information resource management policies. Global Positioning System (GPS) equipment may be used as a component of the information required by the Station Location (SLOC) request process for

creating the certified positional data that will ultimately be entered into the TCEQ's SWQMIS database. In lieu of entering certified GPS coordinates, positional data will be acquired with a GPS and verified with photo interpolation using a certified source, such as Google Earth or Google Maps. The verified coordinates and map interface can then be used to develop a new station location.

BST Analysis

Laboratory Data

Once the samples are received at B-PUB, samples are logged and stored at 4ºC until processed. The COC will be checked for number of samples, proper and exact ID number, signatures, dates, and type of analysis specified. UTB will be notified if any discrepancy is found and proper corrections made. The COC and accompanying sample bottles are submitted to the B-PUB analyst, with relinquishing and receiving personnel both signing and dating the COC. Processed samples will be stored at B-PUB at 4^{o}C until shipment of samples to UTSPH-EP is arranged. Samples will be transported with COC, with relinquishing and receiving personnel both signing and dating the COC. All COC and bacteriological data will be manually entered into an electronic spreadsheet. The electronic spreadsheet will be created in Microsoft© Excel software on an IBM-compatible microcomputer with a Windows® operating system. The project spreadsheet will be maintained on the computer's hard drive, which is also simultaneously saved in a network folder. Data manually entered in the database will be reviewed for accuracy by the UTSPH-EP Project Co-Lead or TWRI PM/QAO to ensure that there are no transcription errors. Hard copies of data will be printed and housed in the laboratory for a period of five years. Any COCs and bacteriological records related to QA/QC of bacteriological procedures will be housed at the UTSPH-EP. All pertinent data files will be backed up monthly on an external hard drive. Current data files will be backed up on an external hard drive monthly and stored in separate area away from the computer. Original data recorded on paper files will be stored for at least five years. Electronic data files will be archived to CD after approximately the end of the project, and then stored with the paper files for the remaining 4 years.

Data Validation

Following review of laboratory data, any data that is not representative of environmental conditions, because it was generated through poor field or laboratory practices, will not be submitted to the TSSWCB. This determination will be made by the UTSPH-EP Project Co-Lead, TWRI PM/QAO, TSSWCB QAO, and other personnel having direct experience with the data collection effort. This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the checks specified in Table D2.1.

Data Dissemination

At the conclusion of the project, the UTSPH-EP Project Co-Lead will provide a copy of the complete project electronic spreadsheet via recordable CD media to the TSSWCB PM, along with the final report. The TSSWCB may elect to take possession of all project records. However, summaries of the data will be presented in the final project report.

Section C1: Assessments and Response Actions

The following table presents types of assessments and response actions for data collection and analysis activities applicable to the QAPP and all facets of the project.

Table C1.1: Assessments and Response Actions

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status monitoring oversight	Continuous	UTB, B- PUB, TWRI	Monitor project status and records to ensure requirements are being fulfilled. Monitoring & review performance & data quality	Report to TSSWCB in QPR
Equipment testing	As needed	UTB, B-PUB, UTSPH-EP	Pass/Fail equipment testing	Repair or replace
Data completeness	As needed	UTB, B-PUB, UTSPH-EP	Assess stations sampled vs. planned sampling	Revisit site or amend objectives
Laboratory inspections	TBD by TSSWCB	TSSWCB	Analytical and quality control procedures in the lab	30 days to respond to TSSWCB with corrective actions
Technical systems audit	As needed	TSSWCB	Assess compliance with QAPP; review facility and data management as they relate to the project	30 days to respond to TSSWCB with corrective actions
Monitoring Systems Audit	TBD by TSSWCB	TSSWCB	Assess compliance with QAPP; review field sampling and data management as they relate to the project	30 days to respond to TSSWCB with corrective actions

In-house audits of data quality and staff performance to assure that work is being performed according to standards will be conducted by all entities. Audits will be documented in a written laboratory journal and initialed by the Project Lead or PM of each respective entity. If audits show that the work is not being performed according to standards, immediate corrective action will be implemented and documented in the laboratory journal.

The TSSWCB QAO (or designee) may conduct an audit of the field or technical systems activities for this project as needed. Each entity will have the responsibility for initiating and implementing response actions associated with findings identified during the on-site audit. Once the response actions have been implemented, the TSSWCB QAO (or designee) may perform a follow-up audit to verify and document that the response actions were implemented effectively. Records of audit findings and corrective actions are maintained by the TSSWCB PM and TWRI QAO. Corrective action documentation will be submitted to the TSSWCB PM with the QPR. If audit findings and corrective actions cannot be resolved, then the authority and responsibility for terminating work is specified in agreements or contracts between participating organizations.

Corrective Action Process for Deficiencies

Deficiencies are any deviation from the QAPP, SWQM Procedures Manual, SOPs, or Data Management Reference Guide. Deficiencies may invalidate resulting data and may require corrective action. Corrective action may include for samples to be discarded and re-collected. Deficiencies are documented in logbooks, field data sheets, etc. by field or laboratory staff. It is the responsibility of each respective entity's Project Leader or PM, in consultation with the TWRI QAO, to ensure that the actions and resolutions to the problems are documented and that records are maintained in accordance with this QAPP. In addition, these actions and resolutions will be conveyed to the TSSWCB PM both verbally and in writing in the project QPRs and by completion of a CAR. All deficiencies identified by each entity will trigger a corrective action plan.

Corrective Action

Corrective Action Reports (CARs) should:

- Identify the problem, nonconformity, or undesirable situation
- Identify immediate remedial actions if possible
- Identify the underlying cause(s) of the problem
- Identify whether the problem is likely to recur, or occur in other areas
- Evaluate the need for Corrective Action
- Use problem-solving techniques to verify causes, determine solution, and develop an action plan
- Identify personnel responsible for action
- Establish timelines and provide a schedule
- Document the corrective action

The status of CARs will be included with quarterly progress reports. In addition, significant conditions (e.g., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported to the TSSWCB immediately.

The Project Lead or PM of each respective entity is responsible for implementing and tracking corrective actions. Records of audit findings and corrective actions are maintained by the Project Lead or PM of each respective entity. Audit reports and corrective action documentation will be submitted to the TSSWCB with the QPR.

Section C2: Reports to Management

Quarterly progress reports developed by the PM and Project Co-Leaders will note activities conducted, items or areas identified as potential problems, and any variations or supplements to the QAPP. CAR forms will be utilized when necessary (Appendix A). CARs will be maintained in an accessible location for reference by all project personnel at TWRI and disseminated to individuals listed in section A3. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP.

If the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problem. CARs will be filled out to document the problems and the remedial action taken. Copies of CARs will be included with the project's QPRs. These reports will discuss any problems encountered and solutions made. These reports are the responsibility of the QAO and the PM and will be disseminated to individuals listed in section A3.

Activities under each task (Surface Water Quality Monitoring, Source Survey, BST Analysis) will be reported in the project final report. This report will then be hosted on the project website.

Section D1: Data Review, Validation and Verification

Surface Water Quality Monitoring

All field and laboratory data will be reviewed and verified for integrity and continuity, reasonableness, and conformance to project requirements, and then validated against the project objectives and measurement performance specifications which are listed in Section A7. Only those data which are supported by appropriate quality control data and meet the measurement performance specifications defined for this project will be considered acceptable, and will be reported to the TSSWCB for entry into SWQMIS.

BST Analysis

All data obtained from field and laboratory measurements will be reviewed and verified for conformance to project requirements, and then validated against the DQOs which are listed in Section A7. Only those data which are supported by appropriate QC data and meet the DQOs defined for this project will be considered acceptable. This data will be submitted to the TSSWCB.

The procedures for verification and validation of data used in BST analysis are described in Section D2. The UTSPH-EP Project Co-Lead is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The TWRI PM/QAO will be responsible for ensuring that all data are properly reviewed and verified, validated, and submitted in the required format as described by the TSSWCB PM. Finally, the TWRI PM/QAO is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

Section D2: Validation Methods

Surface Water Quality Monitoring

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7 of this document.

Data review, verification, and validation will be performed using self-assessments and peer and management review as appropriate to the project task. The data review tasks to be performed by field and laboratory staff are listed in the first two columns of Table D2.1, respectively. Potential errors are identified by examination of documentation and by manual *(or computer-assisted)* examination of corollary or unreasonable data. If a question arises or an error is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented. If an issue cannot be corrected, the task manager consults with the higher level project management to establish the appropriate course of action, or the data associated with the issue are rejected and not reported to the TSSWCB for submission to TCEQ for storage in SWQMIS. Field and laboratory reviews, verifications, and validations are documented.

Table D2.1. Data Review Tasks

Data to be verified	UTB	B- PUB	UTSPH-EP	TWRI
Sample documentation complete; samples labeled	L	LM	L	PM
Field QC samples collected for all analytes as prescribed in TCEQ SWQM Procedures Manual	L			
Standards and reagents traceable		LM	L	
Chain of Custody complete/acceptable	L	LM	L	PM
NELAP Accreditation is current		LM	L	PM
Sample preservation and handling acceptable	L	LM	L	
Holding times not exceeded	L	LM	L	
Collection, preparation, and analysis consistent with SOPs and QAPP	L	LM	L	PM
Instrument calibration data complete	L	LM	L	PM
QC samples analyzed at required frequency	L	LM	L	PM
QC results meet performance and program specifications	L	LM	L	PM
Analytical sensitivity (Min AWRLs) consistent with QAPP	L	LM	L	PM
Results, calculations, transcriptions checked	L	LM	L	PM
Laboratory bench-level review performed		LM	L	
All laboratory samples analyzed for all parameters		LM	L	
Nonconforming activities documented	L	LM	L	PM

Outliers confirmed and documented; reasonableness check performed	L		L	PM
Data properly formatted for SWQMIS inclusion and checked for errors	L			PM
10% of data manually checked	L			PM

L: Leader LM: Lab Manager PM: Project Manager:

After the field and laboratory data are reviewed, another level of review is performed once the data are combined into a data set. This review step as specified in Table D2.1 is performed by the TWRI Project/Data Manager and QAO. Data review, verification, and validation tasks to be performed on the data set include, but are not limited to, the confirmation of laboratory and field data review, evaluation of field QC results, additional evaluation of anomalies and outliers, analysis of sampling and analytical gaps, and confirmation that all parameters and sampling sites are included in the QAPP.

The Data Review Checklist (See Appendix E) covers three main types of review: data format and structure, data quality review, and documentation review. The Data Review Checklist is transferred with the water quality data submitted to the TSSWCB to ensure that the review process is being performed.

Another element of the data validation process is consideration of any findings identified during the monitoring systems audit conducted by the TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. After the data are reviewed and documented, the TWRI PM validates that the data meet the data quality objectives of the project and are suitable for reporting to TSSWCB and subsequently TCEQ.

If any requirements or specifications of the QAPP are not met, based on any part of the data review, the responsible party should document the nonconforming activities and submit the information to the TWRI PM with the data. This information is communicated to the TSSWCB by the TWRI in the Data Summary (See Appendix E).

BST Analysis

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7. The staff and management of the respective field, laboratory, and data management tasks are responsible for the integrity, validation and verification of the data each task generates or handles throughout each process. The field and laboratory tasks ensure the verification of raw data, electronically generated data, and data on COC forms and hard copy output from instruments.

Verification, validation and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the

manager of the task. The data to be verified (listed by task in Table D2.1) are evaluated against project specifications (Section A7) and are checked for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TWRI PM/QAO to establish the appropriate course of action, or the data associated with the issue are rejected.

The UTSPH-EP Project Co-Lead, with assistance from the TWRI PM/QAO, is responsible for validating that the verified data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data for anomalies. The UTSPH-EP Project Co-Lead may designate other experienced water quality experts (TWRI PM or UTB Project Co-Lead) familiar with the waterbodies under investigation to perform this evaluation. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data, before data validation can be completed.

A second element of the validation process is consideration of any findings identified during the monitoring systems audit conducted by the TWRI PM/QAO or TSSWCB QAO. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the TWRI PM/QAO validates that the data meet the DQOs of the project and are suitable for reporting to the TSSWCB.

Section D3: Reconciliation with User Requirements

Surface Water Quality Monitoring

Data produced in this project, and data collected by project personnel will be analyzed and reconciled with project data quality requirements. Data meeting project requirements will be used in the update of the Arroyo Colorado WPP and will be submitted to TCEQ assessment purposes and use in the development of the biennial *Texas Integrated Report for Clean Water Act Sections 305(b) and 303(d)* in accordance with TCEQ's *Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data.* Data which do not meet requirements will not be submitted to SWQMIS nor will be considered appropriate for any of the uses noted above.

BST Analysis

Data produced by this project will be evaluated against the established DQOs and user requirements to determine if any reconciliation is needed. Reconciliation concerning the quality, quantity or usability of the data will be reconciled with the user during the data acceptance process. CARs will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the DQOs of the project and the informational needs of water quality agency decision-makers and watershed stakeholders.

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only BST data that has met the DQOs described in this QAPP will be reported and included in the final project report. Data and information produced thru this project will provide needed information pertaining to watershed characteristics, potential sources of pollution and will aid in the selection of BMPs to address identified water quality issues. Ultimately, stakeholders will use the information produced by this project for the development of appropriate measures to address water quality concerns in the study area. Information produced by this project will be for watershed decisions; namely the update of the WPP.

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APPENDIX A

Corrective Action Report

Corrective Action Report
SOP-QA-001
CAR #:_____

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

State the nature of the problem, nonconformance or out-of-control situation:
_____
_____
_____
_____
_____

Possible causes:
_____
_____
_____

Recommended Corrective Actions:
_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:
_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Program Manager:_____

TWRI Quality Assurance Officer:_____

TSSWCB Quality Assurance Officer:_____

APPENDIX B

Surface Water Quality Monitoring Field Data Sheet

Surface Water Quality Monitoring
Field Data Sheet

The University of Texas at Brownsville
80 Fort Brown - MO1.114
Brownsville, TX 78520
956-882-5938

Sample Location: _____

Station ID: _____ **Data Collected: _____**

Sample Matrix: Water **Time Collected: _____**

Collector(s) Name/Signature:_____

Sample Type: Routine **Sample Depth: _____**

Field Tests and Measurements			Parameters Collected	
	pH (Standard Units)	**00400**		***E. coli* (modified mTEC)**
	Water temperature °C	**00010**		***Enterococcus* (IDEXX)**
	Dissolved Oxygen (mg/L)	**00300**		**Field Split**
	Specific Conductance (µS/cm)	**00094**		
	Instant. Stream Flow (cfs)	**00061**		
Field Observations				
	01351 – Flow Severity (1 – no flow, 2 – low, 3 – normal, 4 – flood, 5 – high, 6 - dry			
	89835 – Flow measurement method (1–gage, 2–electric, 3–mechanical, 4–weir/flume, 5–Doppler)			
	72053 – Days since last significant rainfall			
	89966 – Present weather (1 – clear, 2 – partially cloudy, 3 – cloudy, 4 – rain, 5 – other)			
	74069 – stream flow estimate (cfs) *Required measurements to calculate flow estimates			
	Stream width (feet)*		**Note: Instantaneous stream flow is preferable to stream flow estimate**	
	Average depth of stream (feet)*			
	Distance object travels (feet)*			
	Time for object to travel distance (seconds)*			
Comments				

APPENDIX C

Chain of Custody Record

Sheets of Lading for Fecal Specimen Transport Template

Project Name: Bacterial Source Tracking to Support Adaptive Management of the Arroyo Colorado Watershed Protection Plan				Collecting Entity: University of Texas at Brownsville, Department of Chemistry and Environmental Sciences 80 Fort Brown - MO1.114 Brownsville, TX 78520 Contact: Jude Benavides			Requested Analysis		Container Types: SP: Sterile Polyethylene container Preservatives: 1. Ice 2. H2SO4 3. Sodium Thiosulfate
Name and Signature of Collector:				Receiving Entity: Brownsville PUB – Analytical Laboratory P.O. Box 3270 Brownsville, TX 78523 Contact: Lee Roy Atkinson					Sample Receipt Notice: Ok: All samples 100% correct (labels, COCs, shipping) 1. Not on ice 2. Incorrectly labeled 3. COC incorrect 4. Bottle leaking 5. Other (describe)
Station ID	Sample ID	Media Code	Sample Type	Collection Date	Time	# of Containers	*E. coli*	*Enterococcus*	Sample Receipt Notice:
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
		Water	Routine						
Relinquished by:(print name)		Date	Time	Received by: (Print Name)		Date	Time		Laboratory ID #
1.									Comments
2.									
3.									

CHAIN OF CUSTODY RECORD

Project:	**Remarks:**
Arroyo Colorado BST	**Brownsville PUB – Analytical Lab to UTSPH-EP Lab**
Name and signature of collector:	**Air bill #**

Station ID	**Sample ID**	**Media Code**	**Sample Type**	**Preservative**	**Collection Date**	**Time**

Relinquished by **Brownsville PUB – Analytical Lab**	Date:	Time:	**Received for UTSPH-EP by:**	Date:	Time:

Laboratory Notes:

Media Code: (**FW**) Fecal Isolate from Water Sample; (**FF**) Fecal isolate from Feces; (**FS**) Fecal Sample; (**SS**) Sewage Sample

C-2: Sheets of Lading for Fecal Specimen Transport

(Collector's Organization)

Arroyo Colorado Project

(Collector's Name and title)
(Collector's Phone Number)

In case of EMERGENCY:
(Contact name and number)

Date:_____Time:_____

Sample: Fecal **Hazard:** Bacteria

Species/ Animal:_____

Photo: Yes No

GPS (or other location note): Lat_____Long_____

_____

Other Info:_____

Technician:_____

APPENDIX D

BST STANDARD OPERATING PROCEDURES

D-1: Laboratory Protocol for Isolation and Confirmation of *Escherichia coli* From Water Samples

1. Follow the EPA Method 1603 Modified mTEC procedure (EPA-821-R-02-023, Modified EPA Method 1603; http://www.epa.gov/nerlcwww/1603sp02.pdf).

2. After the Modified mTEC 44.5±0.2°C incubation, the plates should be immediately stored at 4°C until shipment to prevent growth of non-*E. coli* coliforms on the plates.

3. Plates with red or magenta colored colonies should be parafilmed or taped closed, placed in plastic bags and then secured with tape to prevent the plates from being disturbed during shipment.

4. Ship plates in insulated coolers with ice packs sufficient to keep the plates between 1–4°C and ship by next day courier to:

   Dr. George D. Di Giovanni
   UT-Houston School of Public Health
   Biology B224
   500 W. University Ave.
   El Paso, TX 79968
   915-747-8509

5. Presumptive *E. coli* from the Modified mTEC plates will be isolated and confirmed on NA-MUG as described in the protocol for fecal specimens.

D-2: Collection of Fecal Samples for Bacterial Source Tracking

1. Only fresh fecal samples of known origin should be collected. Specifically, fecal samples should be obtained in one of five ways:
    a. Collected from animals visually observed defecating by technician.
    b. Collected from trapped animals.
    c. Collected from intestines of animals legally harvested.
    d. Collected from the intestines of animals recently killed by cars (within 24 hours).
    e. Human (wastewater) samples collected from individual septic tanks, composite septic samples from pump trucks, from wastewater treatment plant influent (for plants with secondary disinfection or lagoon treatment), or from lagoon treatment effluents.

2. Samples should be carefully collected to avoid contamination. Samples on the ground should be collected with a sterile spatula, or similar device, while avoiding collection of material in contact with soil or other possible sources of contamination. Intestinal samples should be collected from animals by using sterile loops inserted anally or by cutting into the intestine using a sterile scalpel. Wastewater samples can initially be collected with sterile bottles, or other suitable device and then transferred to the fecal tubes described below.

3. Each fecal sample should be placed in a new, sterile fecal tube (Sarstedt, cat# 80.734.311). Tubes should be filled approximately ¾ full (can provide less material for smaller animals)

4. Samples should be refrigerated (~4°C) or kept on ice following collection.

5. At the time of sampling, record detailed information on the tube regarding the sample including:
    a. Sampling date
    b. Sampling time
    c. Animal species (common name ok)
    d. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
    e. Sample collector's name/initials
    f. Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle

6. Notify the lab via email or phone (see below) as soon as possible (prior to or immediately following sample collection) with an estimated number of samples that will be shipped and the expected date of shipment. This will allow lab to make appropriate preparations to process the samples immediately upon arrival.

Elizabeth Casarez or George Di Giovanni
elizabeth.a.casarez@uth.tmc.edu or
george.d.digiovanni@uth.tmc.edu
915 747-8076 or 915 747-8509

7. Samples should be shipped (at 4ºC) as soon as possible (within **3 days**) to the lab (address below). 'Blue-ice' or freezer blocks should be used to keep the samples cool, but not frozen during transport. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.

8. Notification of shipment should be sent to the appropriate lab via email or phone (see contact info above) no later than the day of overnight shipping. Notification should include tracking number and direct collections contact person for confirmation upon receipt of samples.

9. Ship samples (and COCs) in insulated coolers (marked on outside to indicate that contents are perishable) with sufficient ice packs to maintain ~4°C to:

George Di Giovanni
UT-Houston School of Public Health
Biology Building B224
500 W. University
El Paso, TX 79968
915-747-8509

D-3: Laboratory Protocol for Isolation and Confirmation of *Escherichia coli* from Fecal Specimens

Note: All collection and handling of fecal specimens should be performed using protective gear (e.g. latex or nitrile gloves). Specimens should be handled aseptically to ensure sample quality and minimize exposure of personnel to pathogens. All feces collected will be placed in screw capped sterile containers. Containers will be labeled with: Name of collector, date, species, GPS location, and photo of specimen before collection. Containers will then be placed in ziplock biohazard bags with lading pouch. Information will be written on lading report and placed in the bag. Fecal specimens will be placed in an insulated cooler on ice during transport to the AgriLife-TP lab. All fecals should be cultured within 24 hours of reaching the lab.

Note: All handling of fecal specimens and cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens.

1. Using a bacteriological loop, streak a loop full of fecal material onto a labeled modified mTEC agar plate (USEPA-821-R-02-023, Modified USEPA Method 1603; http://www.epa.gov/nerlcwww/1603sp02.pdf) for isolation of *E. coli* colonies.

2. Incubate the plate inverted at 44.5±0.2°C for 20 to 24 h.

3. Examine the plate for presumptive *E. coli* colonies, which will appear red or magenta colored.

4. Select up to three presumptive *E. coli* colonies and streak each colony for purity onto a labeled nutrient agar MUG (NA-MUG) plate.

5. Invert and incubate plates at 35 to 37°C for 20 to 24 h.

6. Examine the cultures using a **long-wave handheld UV lamp**. If there is a mixture of fluorescent and non-fluorescent colonies, select a well isolated fluorescent colony and streak again onto NA-MUG for purity.

7. At the discretion of the laboratory, additional biochemical tests such as urease, indole, and citrate tests may be performed.

D-4: Archival of *Escherichia coli* Isolates

Note: All handling of cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. These isolates should be from colonies which have been plated for purity several times and lab personnel are confident purity has been achieved.

1. Select a well-isolated colony of purified *E. coli*. (Examine the cultures using a long-wave handheld UV lamp, colonies will fluoresce).

2. Using a bacteriological loop, transfer the colony to a labeled sterile cryovial containing 1 ml of tryptone soy broth (TSB) with 20% reagent grade glycerol.

3. Firmly cap the cryovial and verify that the cells have been resuspended by vortexing for several seconds; then plunge into liquid nitrogen until frozen.

4. Immediately transfer to a cryostorage box and place in -70 to -80°C freezer. Cultures may be stored for several years under these conditions.

5. To recover cultures from frozen storage, remove the cultures from the freezer and place the cryovials in a freezer block. *Do not allow cultures to thaw.*

    a. Using a bacteriological loop, scrape the topmost portion of the culture and transfer to growth medium, being careful not to contaminate the top or inside of the vial. Invert and incubate plates at 35 to 37°C for 20 to 24 h.

    b. Reclose the cryovial before the contents thaw and return to the freezer.

D-5: ERIC-PCR of *Escherichia coli*

1. Select isolated colonies from overnight cultures of *E. coli* isolates on Brain-Heart Infusion (BHI) plates.

2. Transfer colonies using a 1 µl loop to a sterile microfuge tube containing 100 µl of sterile molecular grade water; vortex briefly to suspend cells.

3. Prepare sufficient PCR Master Mix for samples, including one blank per 10 samples to account for volume loss due to repeat pipetting. Prepare Master Mix for each sample as noted below. <u>One full PCR batch on the MJ Research Cycler 48 well-plate will have 46 samples, *E. coli* QC101, and a no template control.</u>

    <u>ERIC-PCR Master Mix – 24 samples + 2 blanks, prepare X 2 for full 48-well plate</u>

MASTER MIX	Amt (uL)	Final Calc	Final Units
dH2O	**819**		
10X PCR buffer I w Mg	**130**	1	X (1.5 mM)
20 mM dNTP	**13**	200	uM each
ERIC Primer Mix	**130**	600	nM each
BSA (30 mg/ml)	**65**	1.5	ug/uL
AmpliTaqGold (Units)	**13**	2.5	Units/rxn

4. Dispense 45 µl of Master Mix for each sample into the appropriate well of PCR plate.

5. Briefly vortex cell suspensions, then add 5 µl of each cell suspension to the appropriate PCR well.

6. Carefully seal plate using an adhesive PCR cover.

7. Load the plate into the thermal cycler and run under the "ERIC-PCR" program with the following cycling conditions:

    a. Initial denaturation at 95°C for 10 min

    b. 35 Cycles:
        i. Denaturation at 94°C for 30 sec
        ii. Annealing at 52°C for 1 min
        iii. Extension at 72°C for 5 min

    c. Final Extension at 72°C for 10 min

8. Store completed reactions at -20°C until analyzed by gel electrophoresis.

9. Prepare a 250 ml, 2% agarose gel using a 500 ml bottle. Add 250 ml of 1 X Tris/Borate/EDTA (TBE) buffer and 5.0 g agarose. Microwave until agarose is fully dissolved, tighten cap and let cool 1 to 2 minutes, then pour agarose into casting tray with 30-tooth, 1 mm thick comb.

10. Allow gel to solidify for approximately 30 minutes on the bench, then without removing comb place in Ziploc bag and solidify overnight in the refrigerator. The next day carefully remove comb, transfer to gel tank in cold room (4°C) containing pre-cooled 1X TBE buffer. Replace TBE in gel tank after it has been used twice.

11. The following items will be needed for electrophoresis:

    100 bp ladder (0.33 µg/10 µl) (1500 µl final, enough for 150 lanes)

200 µl Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µl 100 bp ladder (add reagents below to a full tube of marker)

300 µl 6X ERIC-PCR loading buffer (see recipe below)

150 µl 10X PCR buffer

850 µl molecular grade water

Store in cold room

6X ERIC-PCR Loading Buffer

25 mg bromphenol blue (0.25%)

1.5 g ficoll 400 (15%)

Add molecular grade water to 10 ml, divide into 1 ml aliquots and freeze, the aliquot currently being used can be stored in the cold room

ERIC-PCR Blank

100 µl 10X PCR buffer

200 µl 6X ERIC-PCR loading buffer

900 µl molecular grade water

Store in cold room

Ethidium Bromide Stain (0.5 µg/ml)

1250 ml 1X TBE

62.5 µl ethidium bromide (Sigma, 10 mg/ml)

Store covered at room temp, can use up to 5 times by adding 10 µl ethidium bromide each additional use

12. Mix 10 µl of 6X ERIC-PCR Loading Buffer to each PCR well and mix with pipette tip.

13. Load the gel in the cold room as follows (max. of 23 samples + QC101 + NTC per gel):

a. Load 10 µl of 100 bp ladder (0.33 µg) into the first lane

b. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

c. Load 10 µl of 100 bp ladder (0.33 µg)

d. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

e. Load 10 µl of 100 bp ladder (0.33 µg)

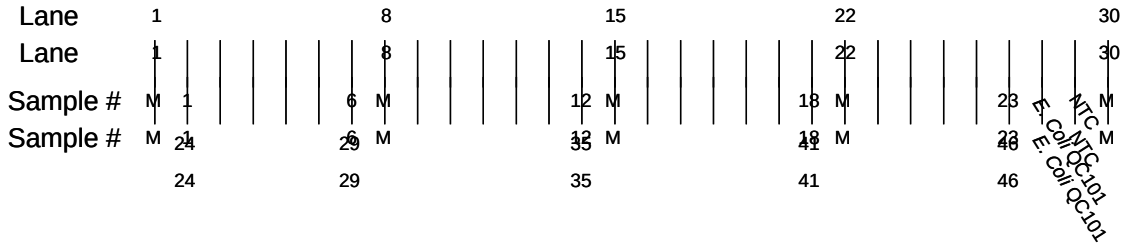
f. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

g. Load 10 µl of 100 bp ladder (0.33 µg)

h. Load 10 µl of sample ERIC-PCR reactions into next 5 lanes

i. Load PCR Batch *E. coli* QC101 and NTC into next 2 lanes

j. Load 10 µl of 100 bp ladder (0.33 µg)



If running a gel with fewer samples, follow steps above until last sample, followed by *E. coli* QC101, NTC and ladder, then load ERIC-PCR Blank into remaining lanes on gel.

14. Start electrophoresis power supply set at 100 volts, run for 1 hour.

15. Stop power supply, set time to “000”, set voltage to 200 and start circulating pump at setting #2, run for 4 hours.

16. After electrophoresis, stain gel in Ethidium Bromide Stain for 20 minutes with agitation (save stain, see Step 13).

17. Destain gel for 10 minutes in 1X TBE buffer. Save destain, can be used 3 times then discard.

18. Follow Gel Logic 200 SOP for image capture. Save digital photograph as a TIFF file (default) and print a hardcopy for notebook.

D-6: RiboPrinting of *Escherichia coli*

Storing and Handling Disposables

Check the lot expiration date on each label for details and rotate the stock to optimize use.

Heating membrane and probe (MP) Base

After storage and the temperature changes that occur during shipment, the oxygen in the buffer loaded in the MP base may need to be removed before use. This is called degassing and is accomplished by heating the base pack overnight in your incubator.

To degas buffer:

1. Place enough MP base packs for the next day's production in their storage pouches in an incubator set at 37°C.

2. Allow the base pack to degas for 16 to 24 hours prior to loading in the characterization unit. You may do this while you are incubating samples, since the base packs are sealed in their pouches. This procedure allows you to start a batch immediately at the beginning of the next shift.

3. If you do not use the heated base packs, you can return them to storage and reuse them. These base packs should be heated again before reuse since temperature cycling affects oxygen content in the buffer.

Preparing Lysing Agent (for *Staphylococcus* and lactic-acid bacteria only)

Lysing agent (A and B) is shipped frozen and must be stored at -20°C. Lysing agent must be thawed before use. This only takes about 5 minutes. If the lysing agent will not be used again for more than 2 hours, the material should be returned to the freezer. Lysing agent can be re-frozen several times with no effect on performance.

Sample Preparation Procedures

1. Incubate and Inspect the Samples

Use BHI agar plates prepared within the last 30 days. Do not use plates that appear dry or dehydrated. Such plates can cause problems when you attempt to "pick" the colonies for use in the RiboPrinter® system.

1. Using a pure isolated colony as the source, streak BHI agar plates heavily in the upper portion of the plate to create a lawn. Streak the remainder of the plate lightly to create single colonies.

2. Follow standard laboratory techniques. Heat plates for 18 to 30 hours in a humidified incubator at 37 °C.

2. Transfer Sample Buffer to Intermediate Tubes

a) Locate the 250 ml twist-top bottle of sample buffer supplied in Pack # 1. Install the twist cap.

b) Transfer about 5 ml of buffer to a sterilized disposable 15 ml intermediate working tube.

3. Add sample buffer to microcentrifuge tubes

1. Place a sterile 0.65 ml microfuge tube in each of the eight holes in the lower row of the sample preparation rack.

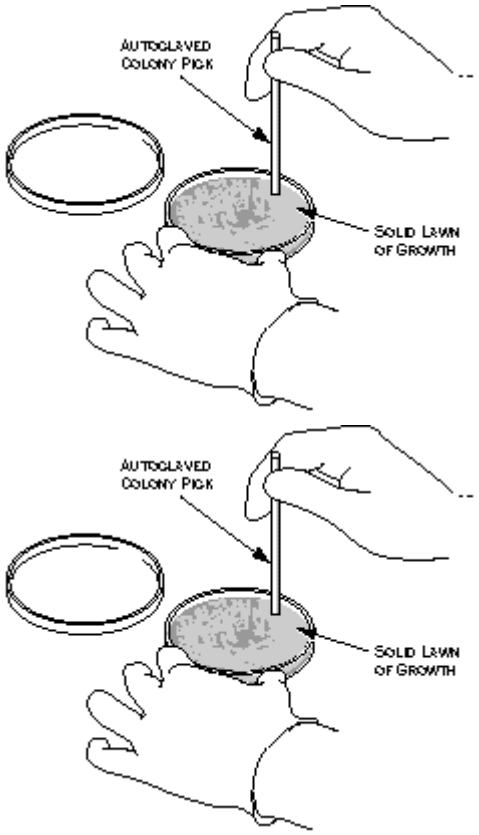
2. For Gram negative samples (including *E. coli*), add 200 µl of sample buffer from the intermediate tube.

   For Gram positive samples (e.g. *S. aureus* and *L. innocua* QC strains), add 40 µl of sample buffer.

3. Close the lids on the tubes.

4. Harvest the Samples

1. Using autoclaved colony picks and making certain not to gouge the agar, carefully place the pick into one of the single colonies or the lawn. You need a sample area at least equal to that of the bottom of the colony pick. In most cases you will need to harvest from the lawn area of the plate. If you are working with large colonies, a single colony will be adequate.



2. <u>For Gram negative samples (e.g. *E. coli*), perform 1 pick placed into 200 µl of sample buffer.</u>

CAUTION! Do not try to use the same pick twice on a plate. You need to harvest only enough sample to cover the bottom surface of the pick. Make sure the end of the pick is flat, if not, use a different pick.

CAUTION! Do not overload the harvesting pick. Collect only enough sample to cover the base of the pick. Over sampling will cause inaccurate results. Over sampling is a particular problem with *Staphylococcus*.

5. Mix the Samples

WARNING! *Perform sample preparation using a Class 2 biological safety cabinet since aerosols may be formed during mixing of the samples.*

1. Making certain not to touch the sample end of the pick, place the pick into one of the filled sample tubes.

2. While holding the tube with the open end facing away from you, carefully attach the pick to the hand-held mixer. The fit of the pick in the coupling will be loose.

WARNING! Do not turn on the mixer unless the pick is inside the sample tube and below the surface of the liquid. Turning the unit on at other times will cause the sample to aerosolize and may cause contamination.

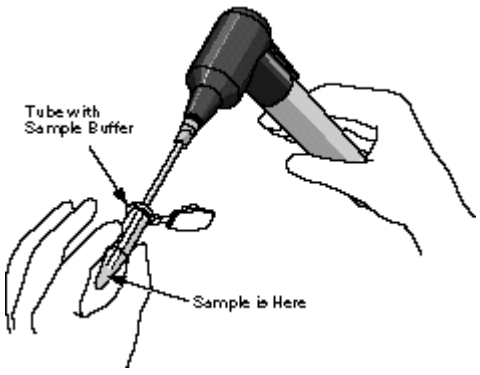
3. Press the ON lever on the mixer for about 5 seconds.

4. Release the lever and carefully remove the colony pick. The sample liquid should appear turbid.

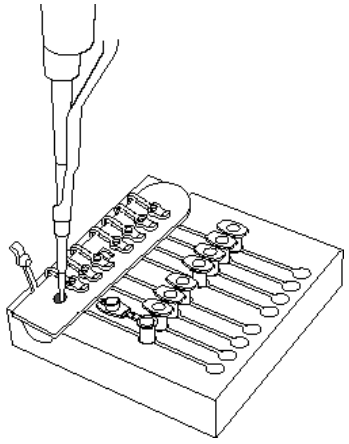
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.

6. Cap the sample tube.

7. Move the tube to the top row of the sample preparation rack. This indicates that the tube is filled.



6. Transfer the Samples to the Sample Carrier



1. Open the lid covering the first well of the sample carrier.

2. Using a 100 µl pipetter, pipette 30 µl of sample from the microcentrifuge tube into the well.

3. Close the lid cover for the well.

4. Repeat for remaining samples using a new pipet tip for each sample.

CAUTION! Transfer the sample carrier to the Heat Treatment Station within 2 hours. If you wait longer than 2 hours, you will have to discard the sample carrier and begin again for this batch.

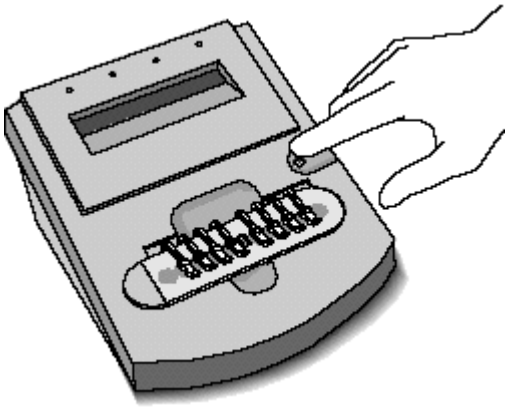
6. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).

7. Write down the name or code you use to identify the sample and the well number in the sample carrier for each sample using a sample log sheet.

7. Place the Sample Carrier in the Heat Treatment Station and Process the Sample Carrier

1. Place the sample carrier into the Heat Treatment Station. The display on the Heat Treatment Station will show **Insert**, if power is available. If the display is blank, make certain that the power cord on the back of the station is properly connected.

   After you insert the carrier, the display shows **Press Button.**



2. Press the button on the Heat Treatment Station.

   The display shows **Warm up** and counts down from **10** while the station is warming up. The actual warm up cycle varies with the condition of the room and the heat treatment station. Normal time is about 4 minutes.

   When the station reaches operating temperature, the display changes to **Heat** and counts down from **13.** This represents each minute of heat treatment.

   The indicator message changes to **Cool.** The display counts down from **9**, indicating the minutes remaining in the cooling cycle**.** If necessary, you can remove the carrier as soon as the **Cool** message appears.

3. The heat treatment step is finished when the display shows **READY** and counts down from **90.** The display will flash and an audible beep will sound three times. The alarm will then beep once every 10 minutes until the sample is removed or 90 minutes elapses.

Caution! The heat-treated samples must be used within the 90-minute period at room temperature or they must be discarded. The heat-treated samples may be stored at this point (prior to adding Lysis Agents, if required) for 1 week at 4 °C, or for several months at -70 °C.

8. Add the Lysing Agents (for *Staphylococcus* and lactic-acid bacteria only)

1. Using a 10-µl pipetter and new tips for each addition, add 5 µl of Lysing Agents A and B to each sample. Note: this step is omitted for *E. coli* as it has no effect on ribopatterns. Lysing Agents were specifically developed for *Staphylococcus* and Lactic-Acid bacteria samples.

Caution! This step must be performed just prior (within 10 minutes) of loading the samples into the RiboPrinter and starting the run.

Creating and Loading a Batch

There are three options under the Operations menu for creating standard batches:

- *EcoR*I batches (VCA)
- *Pst*I batches (VCB)
- *Pvu*II batches (VCC)

You can also create special batches:

- Restriction Enzyme Flexibility batches
- **Substitute Enzyme batches (including *Hin*d III)**

From the Instrument Control Base Window:

1. Move the pointer to Operations and click with the mouse button. The Operations menu appears.

2. Move the pointer to Create Substitute Enzyme Batch and click with the mouse button.

3. Use the View menu to remove any optional items you do not wish to fill in. The system requires at least Sample Type and RiboGroup Library information for each sample. You cannot remove these options. The **Clear** option de-selects the **Use Default ID Libraries.** You will have to enter a DuPont ID and Custom ID library name for all samples. These become required fields and the system will make you enter data before you can save the information in this window.

CAUTION! If you change the display after you have entered information, you will lose all the information in the window. The window will redraw with a new blank display showing the items you have selected.

4. To enter information about the sample, click on the **View** button with the mouse button, and then click on **Sample Items**. Click on the options you want to display.

5. Enter your initials and any comment you want to record about the batch.

6. Select the lot number fields and record for all reagents.

CAUTION! All fields must be completed or the system will not let you start processing the batch.

7. For each well in the sample carrier, choose the type (Sample or Control [QC Number]) from the Sample Type field. The system defaults to Sample.

8. Once you define the Sample Type as Sample, type in the name you actually want to use. This information will appear as Sample Label in the Data Analysis software screens.

9. You can change the RiboGroup library name if needed. Do this by clicking on the button next to the field with the mouse button. A pop up menu appears listing your choices. If you want to add a new library name, move the pointer to the line and click with the mouse button to get a cursor, then type in the new library name. Once you have saved this file, the new name will be added to the pop up list for future use. Do **NOT** change the DuPont ID field. If you select one of the QC strains, the system automatically enters QC in the DuPont ID and RiboGroup Library fields. Do not change these names. If you wish, you may enter a name for the Custom ID library.

10. Repeat for the other seven samples.

11. Click on Save and Submit Batch to Instrument.

Loading Disposables

Follow the screen prompts to load disposables and check the DNA Prep Waste. The icons on the window will flash red to tell you to remove and load an item. The screen prompts you about which Separation and Transfer chamber to use for the membrane and gel cassette. The LDD Pipette will move to physically block you from placing samples in the wrong chamber.

CAUTION! *Do not try to move the pipette manually. You will cause the system to lose the step count. This can result in the loss of batch data. If the pipette is blocking the S/T chamber that you are instructed to use, STOP. Call Customer Support.*

CAUTION! *Do not load disposables until you are prompted by the system. If you try to load them earlier, the alarm will sound as long as the doors are open. If you do load disposables ahead of time, the MP Base will be moved to the wrong position and you will not be able to begin processing the batch. You will not be able to move the MP base manually.*

1. Check the DNA Preparation Waste Container

1. The DNA Prep waste container must be visually checked before every batch. If the container looks nearly full (about 1 inch from the top), remove the container, unscrew the cap and empty into the liquid biohazard waste.

WARNING! Do not tip the DNA Preparation waste container when you remove it.

WARNING! Do not unscrew the cap from the DNA Preparation waste container if the fluid level has risen into the cap. First pour the excess waste liquid into the liquid biohazard waste.
WARNING! When replacing container make sure that the cap is properly threaded in place. If the cap is only partially threaded, it can snag the pipette during operation.

2. Load the Sample Carrier

1. Place the sealed carrier into the labeled slot on the far right of the characterization unit.

2. Push the sample carrier down firmly until it snaps into place.

CAUTION! Place the rounded edge of the sample carrier on your right as you view the characterization unit. Position the carrier this way to insure correct identification of the sample wells.

3. Load the DNA Prep Carrier

1. Remove the DNA Prep carrier from the refrigerator.

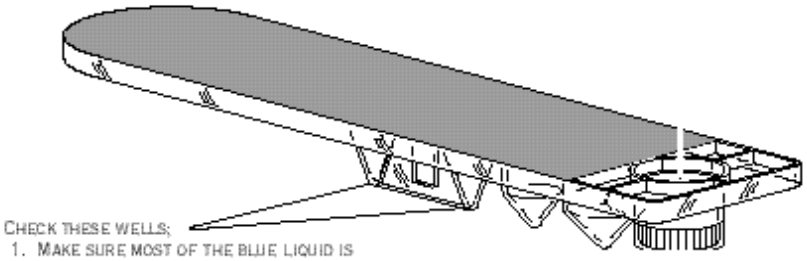
2. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**

3. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.

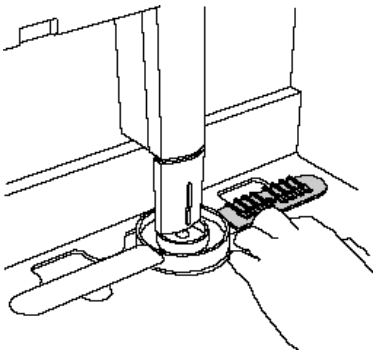
4. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µl microfuge tube (Cat. #72730-005) as a 50 U/µl working stock as follows.**

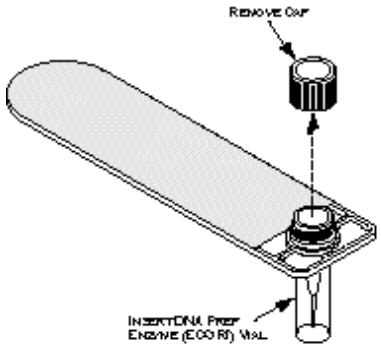
   <u>50 U/µl:</u> 26.5 µl *Hin*d III and 26.5 µl of NEB 10X Buffer 2

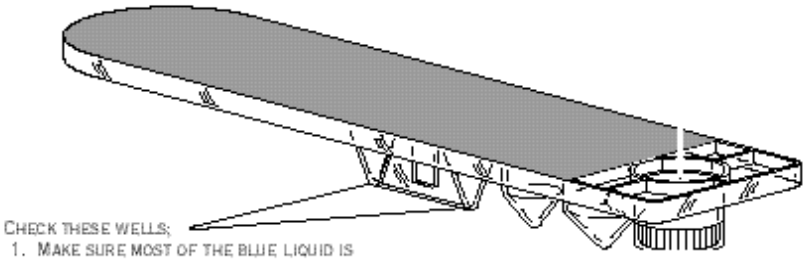
   During addition of the Buffer, mix enzyme and buffer to homogeneity by stirring with the micropipette tip.



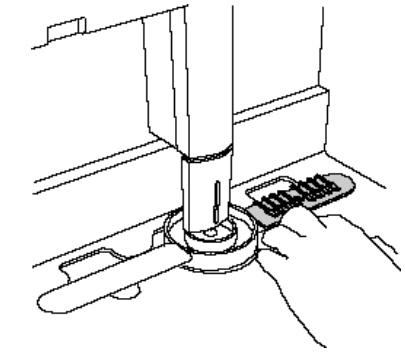
CHECK THESE WELLS;
1. MAKE SURE MOST OF THE BLUE LIQUID IS AT THE BOTTOM.
2. MAKE SURE THERE ARE NO AIR BUBBLES.

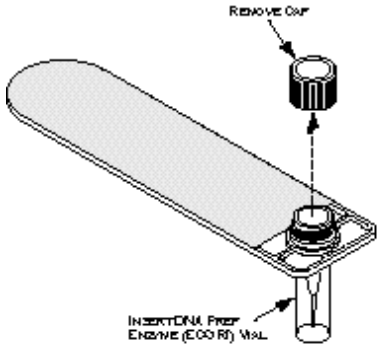






CHECK THESE WELLS;
1. MAKE SURE MOST OF THE BLUE LIQUID IS AT THE BOTTOM.
2. MAKE SURE THERE ARE NO AIR BUBBLES.



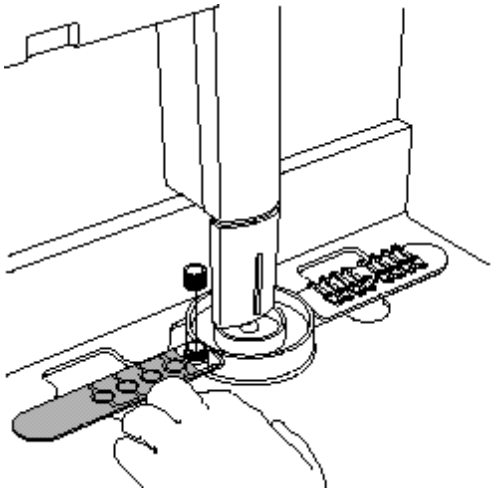


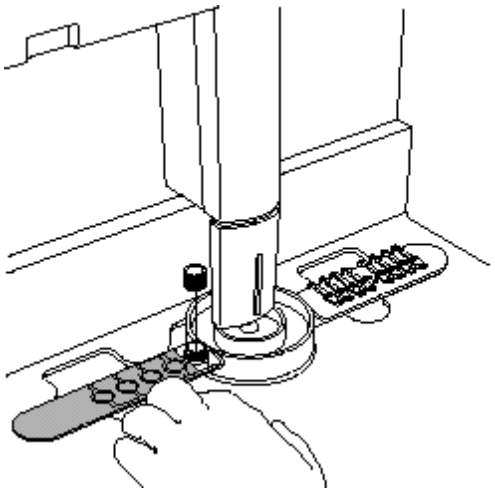
5. Remove the cap from the Enzyme vial.

6. Insert the vial into the carrier.

7. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.

8. Push the DNA Prep carrier down firmly until it snaps into place.



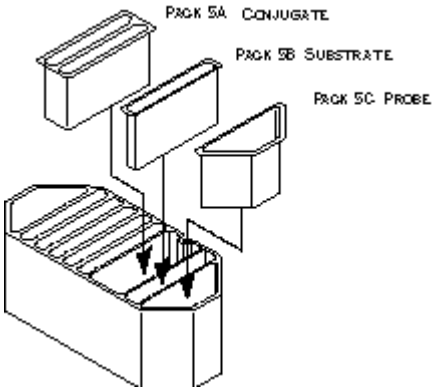


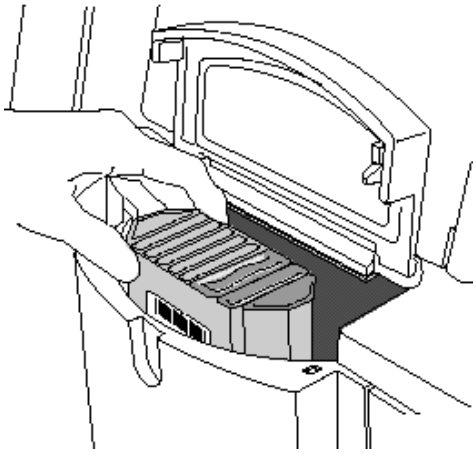
4. Load the MP Base and Carousel

1. Unpack the disposables.

2. Remove the MP base (Pack 5) from the incubator and the Conjugate (Pack 5A), Substrate (Pack 5B), and Probe (Pack 5C) from the refrigerator.

3. Remove each insert from its pouch. Tap the powdered reagent packs gently to bring all powder to the bottom of the packs. Place reagent packs in the MP base and load the base in the carousel.

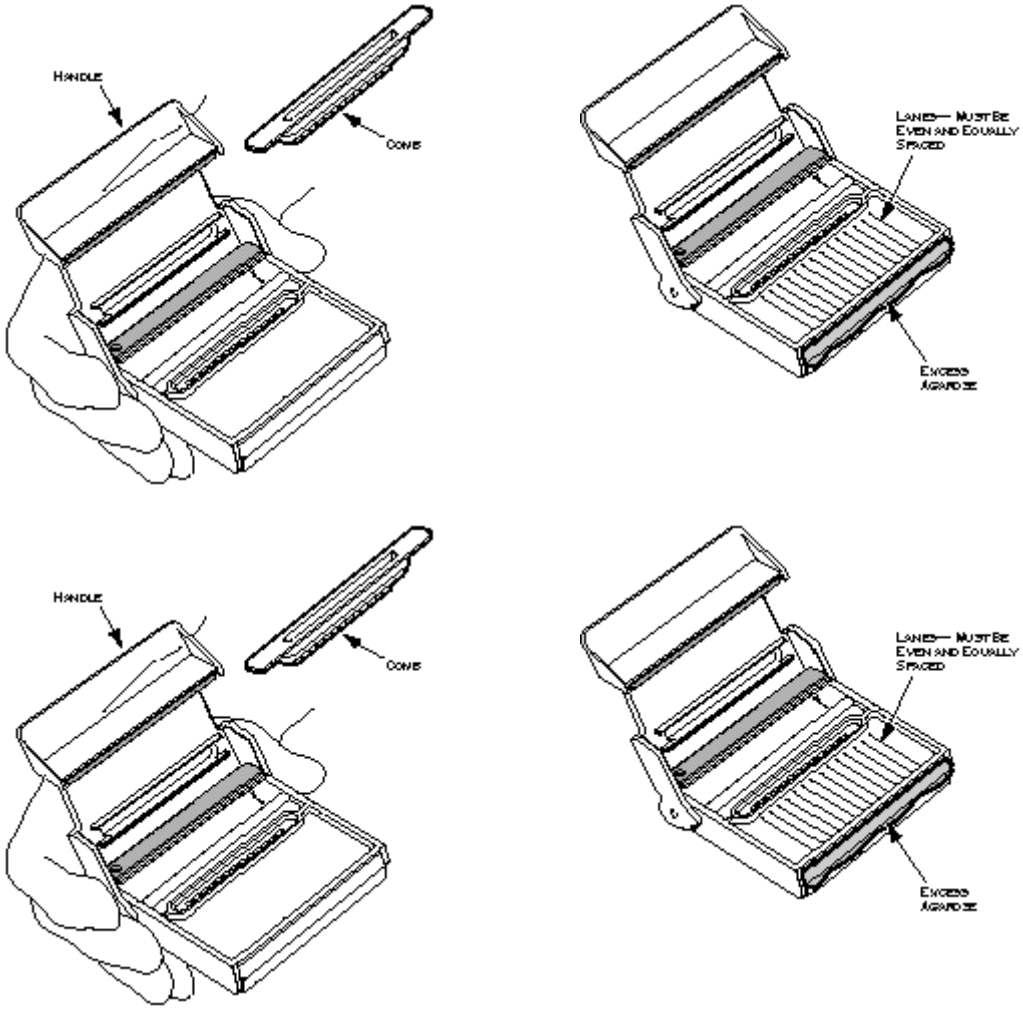




CAUTION! Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.

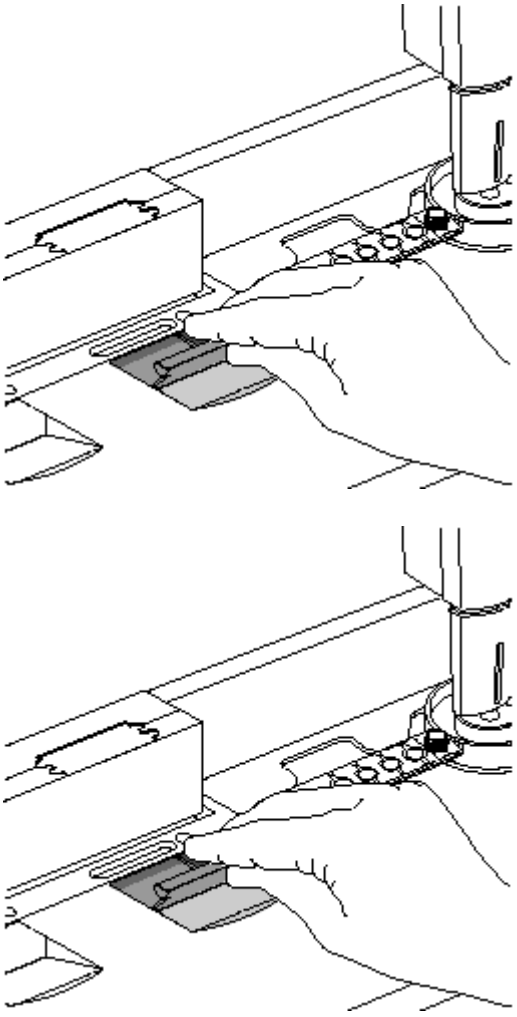
5. Load the Gel Cassette

1. Remove the gel cassette from its package.
2. Grasp one end of the rubber comb and gently pull the comb from the cassette.
3. Unfold the handle of the cassette towards you until the handle snaps into place.
4. Check the front edge of the gel cassette and the lanes of the gel.



Warning! *If the cassette shows a build up of excess gel on the front edge, or if you notice any shrinkage of the gel away from the cassette or bubbles, record the lot number and call Customer Support. Use a new cassette for this run.*

5. Insert the gel cassette into the slot labeled **Gel Bay.** The RiboPrinter® system will prevent the insertion of the cassette into the incorrect slot by blocking one slot with the LDD Pipette.

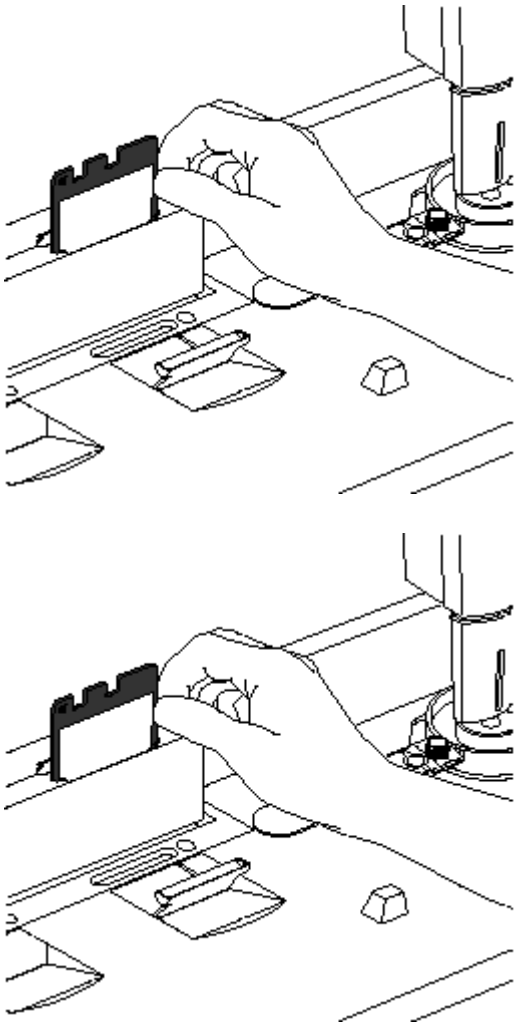


6. Press the cassette forward firmly until it snaps into place.

6. Load the Membrane

1. Grasp the membrane and carefully drop it into the front slot and flip the metal bracket against the back of the membrane.

CAUTION! You can insert the membrane backwards. This will cause an alarm that prevents the sample from being processed until the error is corrected. Always make certain that the two large slots are on top and that the square hole on the side faces your left as you insert the membrane.



7. Close all doors and the instrument will begin sample processing.

8. Load the Next Batch

The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.

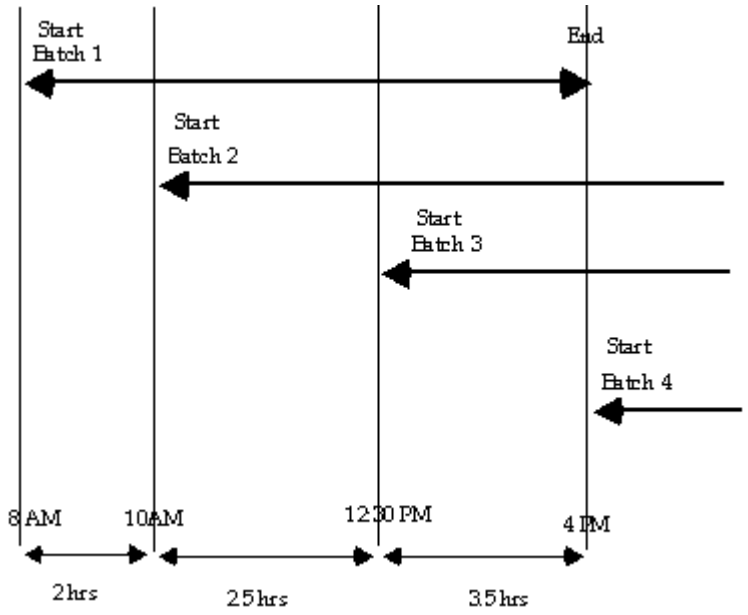
The chart above shows the approximate loading times for each batch in a work shift using only the VCA protocol.

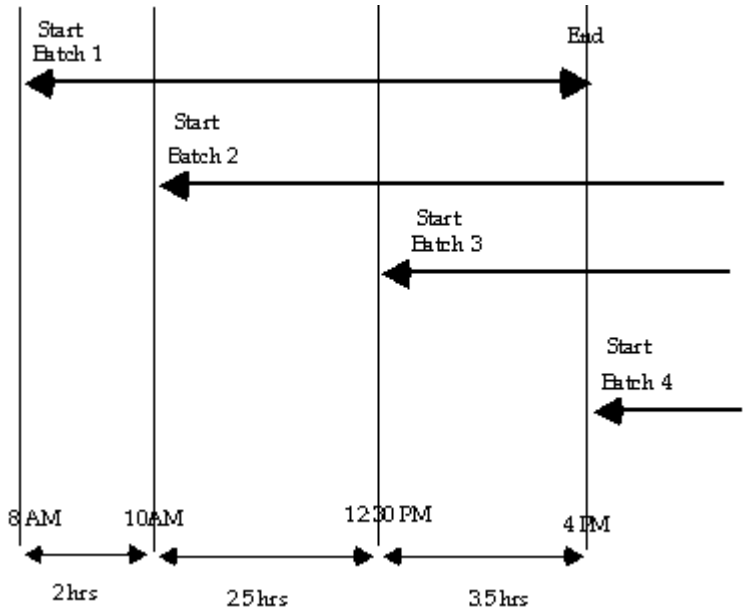
1. You can now use the **Create Batch** option to set up a new pending batch.

2. When you complete the information window and click on the **Start Normal Batch** option, the window displays a message telling you when you can load the next batch.

Batch Report

After image processing is completed, the system automatically runs a series of analysis functions and generates a Batch Information Report. This task does not require any action on the part of the operator. Reports are automatically saved to the hard disk of the computer and sent to the printer.




Start
Batch 1
End
Start
Batch 2
Start
Batch 3
Start
Batch 4
8 AM
10 AM
12:30 PM
4 PM
2 hrs
2.5 hrs
3.5 hrs

APPENDIX E

Data Review Checklist
&
Data Summary Sheet

Data Review Checklist

Title of associated QAPP: _____

✓, X, or N/A

Data Format and Structure

A. Are there any duplicate *Tag ID* numbers? _____
B. Are the *Tag prefixes* correct? _____
C. Are all *Tag ID* numbers 7 characters? _____
D. Are TCEQ station location (SLOC) numbers assigned? _____
E. Are sampling *Dates* in the correct format, MM/DD/YYYY? _____
F. Is the sampling *Time* based on the 24-hour clock (e.g. 13:04)? _____
G. Is the *Comment* field filled in where appropriate (e.g. unusual occurrence, sampling problems, unrepresentative of ambient water quality) and any punctuation deleted?

_____

H. *Source Code 1, 2* and *Program Code* are valid and used correctly? _____
I. Is the sampling date in the *Results* file the same as the one in the *Events* file? _____
J. Values represented by a valid parameter (*STORET*) code with the correct units and leading zeros? _____
K. Are there any duplicate parameter codes for the same *Tag Id*? _____
L. Are there any invalid symbols in the Greater Than/Less Than (*GT/LT*) field? _____
M. Are there any tag numbers in the *Results* file that are not in the *Events* file? _____
N. Have confirmed outliers been identified? (with a "1" in the Verify_flg field) _____
O. Have grab data (bacteria, for example) taken during 24-hr events been reported separately as RT samples? _____
P. Is the file in the correct format (ASCII pipe-delimited text)? _____

Data Quality Review

A. Are all the values reported at or below the AWRL? _____
B. Have the outliers been verified? _____
C. Checks on correctness of analysis or data reasonableness performed?
e.g.: Is ortho-phosphorus less than total phosphorus?
Are dissolved metal concentrations less than or equal to total metals? _____
D. Have at least 10% of the data in the data set been reviewed against the field and laboratory data sheets? _____
E. Are all parameter codes in the data set listed in the QAPP? _____
F. Are all stations in the data set listed in the QAPP? _____

Documentation Review

A. Are blank results acceptable as specified in the QAPP? _____
B. Were control charts used to determine the acceptability of field duplicates? _____
C. Was documentation of any unusual occurrences that may affect water quality included in the Event file Comments field? _____
D. Were there any failures in sampling methods and/or deviations from sample design requirements that resulted in unreportable data? If yes, explain on next page. _____
E. Were there any failures in field and laboratory measurement systems that were not resolvable and resulted in unreportable data? If yes, explain on next page. _____

✓ = Yes **X = No** **N/A** = Not applicable

Describe any data reporting inconsistencies with AWRL specifications. Explain failures in sampling methods and field and laboratory measurement systems that resulted in data that could not be reported to the TCEQ. (attach another page if necessary):

_____

_____

_____

_____

_____

Date Submitted to TCEQ: _____
Tag ID Series: _____
Date Range: _____
Data Source: _____
Comments (attach README.TXT file if applicable):

_____

_____

_____

_____

Planning Agency's Data Manager Signature: _____

Date: _____

DATA SUMMARY

Data Set Information

Data Source: _____.

Date Submitted: _____.

Tag_id Range: _____.

Date Range: _____.

Comments:

Please explain in the space below any data discrepancies discovered during data review including:

- Inconsistencies with AWRL specifications or LOQs
- Failures in sampling methods and/or laboratory procedures that resulted in data that could not be reported to the TCEQ (indicate items for which the Corrective Action Process has been initiated).
- Include completed Corrective Action Plans with the applicable Progress Report.

_____

_____

_____

_____

_____

_____

_____

_____

☐ I certify that all data in this data set meets the requirements specified in Texas Water Code Chapter 5, Subchapter R (TWC §5.801 et seq) and Title 30 Texas Administrative Code Chapter 25, Subchapters A & B.

☐ This data set has been reviewed using the Data Review Checklist.

Planning Agency Data Manager: _____.

Date: _____.